STUDIES ON THE HUMORAL IMMUNE RESPONSE TO FELINE CORONAVIRUS

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The fear of the Lord

is the beginning of knowledge

Proverbs 1:7

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ABBREVIATIONS

A	ampere
ADE	antibody-dependent enhancement
AMPS	ammonium persulphate
AP	alkaline phosphatase
BCIP	5-bromo-4-chloro-3-indolyl phosphate
CCV	canine coronavirus
cm	centimetres
CMI	cell mediated immunity
CNS	central nervous system
CO_2	carbon dioxide
Coomassie	Coomassie Brilliant Blue
Con A	concanavalin A
CPE	cytopathic effect
CVLP	coronavirus like particles
d	days
DIC	disseminated intravascular coagulation
dpm	disintegrations per minute
DSH	domestic short hair
DTH	delayed type hypersensitivity
DW	distilled water
EDTA	ethylenediaminetetra-acetic acid
EGTA	ethyleneglycol-bis-(beta-aminoethyl ether) N,N,N′,N′,-tetraacetic acid
ELISA	enzyme linked immunosorbent assay
EM	electron microscope
ER	endoplasmic reticulum
FBS	foetal bovine serum
FCA	Freund's complete adjuvant
FCoV	feline coronavirus
FCoVV	feline coronavirus-associated vasculitis
fcwf	felis catus whole foetus
FEA	feline embryo A cells
FECV	feline enteric coronavirus
FEL	feline embryonic lung
FeLV	feline leukaemia virus

FIP	feline infectious peritonitis
FIPV	feline infectious peritonitis virus
FITC	fluorescein isothiocyanate
FIV	feline immunodeficiency virus
FVU	Feline Virus Unit
g	gramme (weight), gravity
GIT	gastrointestinal tract
H & E	Haematoxylin & Eosin
HCV 229E	human coronavirus serotype 229E
HRP	horse radish peroxidase
hr(s)	hour(s)
IC	immune complex
IF	Immunofluorescence
IFA	immunofluorescent antibodies
IFN	interferon
Ig	immunoglobulin
IL-1,2,6	interleukin-1,2,6
iu	International units
i/m	intramuscularly
i/p	intraperitoneally
i/v	intravenously
K	kilodaltons
l	litre
m	metre
M	integral membrane glycoprotein, molar
mA	milliamp
MAb	monoclonal antibody
MCH	multicat household
MEM	Minimum Essential Medium
mg	milligram
min	minute
ml	millilitre
mm	millimetre
Mr	relative molecular mass
N	nucleocapsid
NBT	nitro blue tetrazolium
nm	nanometre

OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
p.i.	post infection
PM	post mortem
RSB	reducing sample buffer
RNA	ribonucleic acid
rpm	rotations per minute
S	spike
s/c	subcutaneously
SCH	single cat household
SDS	sodium dodecyl sulphate
SG	specific gravity
SI	small intestine
SPF	specific pathogen free
TBS	tris buffered saline
TEMED	tetraethylmethylene diamine
TGEV	transmissible gastroenteritis virus
TNF	tumour necrosis factor
Tris	tris (hydroxymethyl) amino methane
ug	microgram
ul	microlitre
URT	upper respiratory tract
UV	ultraviolet
V	Volts
VN	virus neutralising
VNA	virus neutralising antibodies
w	weeks
WBC	white blood cells
wk(s)	week(s)

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DECLARATION

The studies described in this thesis were carried out in the Department of Veterinary Pathology at the University of Glasgow Veterinary School between December 1987 and October 1991. The author was responsible for all results except where it is stated otherwise.

No part of this thesis has been presented to any university but it has been reproduced in parts in the following scientific papers:

Addie D.D. 1989 Interpretation of feline coronaviral serology. In Practice. **11** 6 232

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Addie D.D., Jarrett O. A study of naturally occurring feline coronavirus infections in kittens. In press.

SUMMARY

The first objective of this research was to evaluate the use of the immunofluorescent antibody (IFA) test in feline coronavirus (FCoV) infection. As a result of this work, guidelines for veterinary practitioners were established for the use of this test. Some differences between the reality of FCoV infection in the field and much of the present wisdom based on the extrapolation of experimental infections were found.

The second aim was to determine the fate of seropositive cats. Long-term observation of seropositive cats has never previously been attempted, so that whether these cats develop disease, become carriers, remain seropositive or become seronegative was unknown. We were also curious to discover what happened to kittens born to seropositive cats.

Chapter 1 is a review of the relevant literature to be cited later in the thesis and gives the reader an idea of the state of the art prior to this work.

In Chapter 2 the materials and methods which were used in the experiments described in this thesis are given. Additional materials and methods relevant to each chapter are to be found at the beginning of the chapter concerned.

In Chapter 3 an epidemiological survey is described. The prevalence of FCoV antibodies in cats from different backgrounds was determined. The cat most likely to be seropositive was the healthy pedigree (53% were seropositive) and the cat least likely to be seropositive was the healthy domestic (14% seropositive). Cats from multicat households (MCH) were more likely to be seropositive than those from single cat households (SCH).

A picture was formed of the cat most likely to succumb to feline coronaviral vasculitis (FCoVV): 69% of animals were less

than 18 months old and 90% were from MCH. The cats were often pedigree animals and the highest incidence was in Persians. The prevalence of FeLV or FIV was no greater in cats with FCoVV than in the ordinary cat population. Cats with non-effusive FCoVV appeared to have higher antibody titres than those with effusive FCoVV.

Abnormal gross pathology was noted in a number of cats and caused us to re-evaluate our thinking about the nature of the disease and to discard the term feline infectious peritonitis (FIP) in favour of the more descriptive term FCoVV.

Chapter 4 describes a survey of over 700 cats from 72 households in which cats were seropositive for FCoV.

It had been believed that most seropositive households were infected with a non-pathogenic FCoV or feline enteric coronavirus (FECV). However, deaths occurred in households with all kinds of clinical history: those which had had a case of FCoVV, or those which were presumed to have FECV or non-pathogenic FCoVs. The mortality rate was found to be 12% in households in which the infection was thought to have newly arrived and fell to 3-4% in those households and others in which it was long-standing.

Twenty-four of the households became seronegative, though 9 of them became reinfected, largely by the introduction of a new seropositive animal. There was no evidence to suggest that seronegative cats could excrete virus. Loss of antibodies was related to the initial number of seronegative cats within the house, how the cats were housed and if the highest antibody titre in the household was up to 640 the household was more likely to become seronegative.

Cats which went on to die of FCoVV had variable antibody patterns over the weeks and months that they were followed. Only one of 71 cats whose antibody titres rose from less than

or equal to 20 to 320 or over went on to die of FCoVV. However, one of the 96 cats whose IFA titres fell from 320 or over to 20 or less also died of FCoVV. Thus, looking at serial tests from an individual cat does not reveal its prognosis. Neither does it reveal whether it sheds virus unless its titre falls to zero.

Four cats which became seronegative died of microscopic lesions in the kidneys and brain (1 cat). More cats die as a result of FCoV infection than is apparent by looking only at the classical manifestations of peritonitis or granulomata.

There was no evidence of ADE in cats which appeared to be reinfected (i.e. their titres fell 3 or 4 fold and rose again).

Households which had endemic FeLV and FIV lost no more cats to FCoVV than the negative households.

In Chapter 5, the results of the survey of kittens born into the households described in Chapter 4 are presented. The most significant finding was that careful management of the kittens could prevent them from being infected in households where FCoV was endemic. Keeping kittens isolated with their mother considerably reduced the chances of the kittens becoming infected and none of the kittens which were totally isolated from all adults from 5-6 weeks of age seroconverted.

The existence of asymptomatic carrier cats was demonstrated and their IFA titres were found to be variable and unremarkable. Significantly, seronegative cats were shown not to excrete virus. It was also shown that virus excretion was transient in many cats.

In Chapter 6 experiments are described in which sera from cats whose fates were known were immunoblotted.

Many of the sera from cats in the survey described in Chapter 4

were immunoblotted. A discrepancy was shown between the levels of anti-integral membrane glycoprotein (M) antibody in cats which survived compared with cats which developed FCoVV. Cats which became ill appeared to have greater levels of anti-M. However, using immunoblotting, accurate titres could not be obtained. Difficulty was encountered in making reproducible immunoblots due to variability of the M protein entering the gel.

By immunoblotting, many cats which had IFA titres of zero were found to have anti-nucleocapsid (N) antibody which showed that they had, in fact, been infected. Cats with only anti-N antibodies did not appear to excrete virus but it did not follow that cats which also had anti-M and anti-S did excrete virus.

Chapter 7 is a discussion of the results presented in this thesis and compares and contrasts these with previous work based on experimental infections. Future projects making use of the serum samples and data accumulated during this survey are proposed.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

In the early 1960s, veterinary practitioners first described a chronic fibrinous peritonitis in the cat [55]. This condition was recognized to be infectious and the disease was named feline infectious peritonitis (FIP). Soon a coronavirus was isolated as the cause and was given the name feline infectious peritonitis virus (FIPV) [152].

Subsequently, a granulomatous form of the disease was recognised [87] and came to be known as 'dry' or 'non-effusive' FIP, while the initial presentation was dubbed 'wet' or 'effusive' [104]. As more information was gathered the inappropriateness of this nomenclature became clear for two reasons [2, 28, 57]. First, serological surveys revealed that the majority of infections were asymptomatic, [57, 102, 105, 106, 111, 155]. Secondly, in those animals which became ill, peritonitis was only one manifestation of a variety of possible clinical signs [57].

An early term used to describe the disease was feline systemic proliferative and exudative vasculitis [28, 87]. The primary lesion which leads to clinical and pathological signs of disease is a disseminated pyogranulomatous vasculitis [138, 152]. Therefore in this thesis the disease will be referred to as feline coronaviral vasculitis (FCoVV) [2, 27, 28].

It was realised that effusive and non-effusive forms were not two distinct forms but were gradations of the same process. The effusive form is a more acute presentation while the non-effusive FCoVV is an intermediate stage between effusive FCoVV and immunity [88, 112, 114]. The nature of the granulomas in non-effusive FCoVV results from a partially successful attempt

by the host to wall off and contain the infection. In some cats clinical signs progress from effusive to non-effusive forms [28, 111, 114]. Both forms can be present simultaneously: parenchymal, ocular and CNS lesions could be found in 10% of effusive cases [106] and small quantities of fluid were present in non-effusive cases [87, 95].

An explanation advanced for the discrepancy between the serological evidence of high prevalence of infection and low prevalence of disease was that different strains of coronavirus of differing virulence might exist [85, 110, 111]. A strain of FCoV was discovered which was named feline enteric coronavirus (FECV) which did not produce FCoVV even after i/p inoculation [106, 111] and it was postulated that this virus was the cause of the majority of asymptomatic infections or those which produced only diarrhoea [106, 111]. Later field isolates of FIPV proved to be highly infectious but of low FIP- or FCoVV-inducing capacity [114].

Throughout this thesis the term feline coronavirus (FCoV) will be used to cover both FIPV and the later isolated FECV [103, 110, 111] except where differentiation is relevant.

Pedersen's current view is that all FCoV infections are enteric and that FIPV arises as a virulent mutant in each particular cat which develops FCoVV. This mutation is postulated to allow the virus to replicate in macrophages [104, 108, 114].

As well as virus strain virulence, it is likely that dose of virus, route of infection, the manner in which viral peptides are processed by antigen presenting cells, response of T lymphocytes to processed antigen and ability of virus strain to replicate within macrophages are important factors in the type of resulting clinical presentation [27, 106, 114, 138, 141].

1.2 Feline coronavirus

1.2.1 Characteristics of the FCoV particle

FCoV is an enveloped virus with a fringe of surface peplomers like the corona of the sun, from which it derives its name [103,153]. FECV, FIPV and canine coronavirus (CCV) are morphologically indistinguishable [27, 82, 103, 106, 111, 114, 126].

FCoV has been described as a pleomorphic, spherical or elliptical virus with an overall diameter of 70-150 nm. [14, 26, 39, 43, 57, 62, 94, 95, 103, 104, 111]. It has a doughnut shaped nucleoid 50-73 nm in diameter with a central lucent area of around 30 nm [43, 57, 62, 100, 152]. The envelope consists of a trilaminar unit membrane with numerous petal-shaped spike-like projections, 15-21 nm long [14, 43, 57, 62, 95, 100, 104].

FCoV has a reported buoyant density of 1.17-1.18 g/cm^{-3} in sucrose [26, 57, 82, 94, 102, 131] and a sedimentation coefficient of 400S [57].

According to early reports, FCoV is heat-labile, losing infectivity in 24-48 hours at room temperature [5,31]. The virus is inactivated by incubation at 56^{o}C for 60 minutes [100]. However, in recent studies, FIPV was recovered from contaminated dry surfaces for 3-7 weeks [64]. It is relatively stable at 4^{o}C [31].

FCoV is ether-sensitive and is inactivated by most detergents, disinfectants and household bleach (sodium hypochlorite) [5, 9, 39, 100, 104]. FCoV is destroyed in 60 minutes at room temperature in chlorhexidine and benzalkonium chloride in concentrations of over 0.5g/l [100] and by 0.2% formaldehyde or quarternary ammonium compounds but is resistant to 0.7% phenol for over 48 hours at 4^{o}C [5,100].

The coronavirus genome is a positive sense single-stranded RNA

of about 30 kilobases [11, 20, 21, 22]. During replication a negative strand serves as a template for the synthesis of one genome-sized and multiple sub-genomic mRNAs. In virus-infected cells 6 virus-specific mRNAs are produced with sizes ranging from 1.6 to over 20 kilobases [20].

1.2.2 Viral proteins

FCoV contains three polypeptides: spike, nucleocapsid and integral membrane glycoprotein

1.2.2.1. The spike, (S) peplomer, or E2, is a glycoprotein with a Mr of 180-225.5K [15, 18, 19, 21, 22, 30, 65, 73, 146]. The protein moiety of S is 1150-1450 amino acid residues in length and contains an N-terminal signal sequence, a C-terminal transmembrane anchor and 21-35 potential N-glycosylation sites [22]. There is also a 90-95kD protein which may be a monomeric form of the spike [15, 67]. Precursor proteins of 138K and 175K have also been noted [73]. Unlike that of IBV, the S of FCoV has not been reported to be cleaved [22].

The S glycoprotein contains a hydrophilic trans-membrane domain (S_2) and a glycosylated external domain (S_1). The part of the spike which is anchored in the matrix shows 35% amino acid sequence homology with infectious bronchitis virus (IBV), a coronavirus of domestic poultry, and 29% homology with mouse hepatitis virus (MHV). There is very little conservation of the external domain. Most antigenic variability between laboratory strains of FCoV, as determined by panels of MAbs, has been localised to the S glycoprotein [31, 54].

The spike is responsible for binding the virus to cell receptors and for cell membrane fusion [22, 31, 65, 148] and therefore for CPE in cell culture. Antibodies which neutralize virus in vitro are believed to be directed against the spike [31, 65, 148].

The spike also elicits cell-mediated cytotoxicity and causes

pH-dependent thermolability [31].

1.2.2.2. The nucleocapsid (N), or nucleoprotein is a phosphorylated protein which has a Mr of 43-50K [15, 21, 30, 59, 65, 67, 73] and as high as 58K for one isolate [19]. It is associated with the viral RNA. The N is highly conserved among FCoVs [30]. One reported difference between FECV and FIPV was that the Mr of the N protein of the former was 43K and of the latter, 42K [146].

1.2.2.3. The integral membrane glycoprotein, matrix, (M), or E1 glycoprotein commonly forms a diffuse band on PAGE between 23 and 32K [15, 19, 21, 30, 58, 59, 65, 73, 146]. The Coronavirus Study Group recommended that this protein should no longer be referred to as matrix because its function is different from that of the matrix proteins of para- and orthomyxoviruses [18]. The sequence of the M protein is highly conserved among FCoV isolates [29,30]. It is one of the few viral glycoproteins with O-linked glycosylation [65].

1.2.3 Related coronaviruses

FIPV and FECV belong to the same taxonomic cluster of coronaviruses as transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus, canine coronavirus (CCV), human bronchitis coronavirus serotype 229E (H 229E) and coronavirus-like particles (CVLP) [4, 11, 30, 57, 66, 82, 94, 95, 104, 106, 122, 123, 127, 140]. Laboratory strains of FCoV have been classified into types I and II according to their growth characteristics in cell-culture, pathogenicity and their comparative neutralization by antisera to various strains of FECV, FIPV, TGEV and CCV [31, 113].

Antigenically, FECV, FIPV and CCV are virtually indistinguishable, [15, 27, 30, 66, 67, 82, 96, 103, 106, 114, 126]. Investigation with polyclonal and monoclonal antibodies indicates that FIPV type II and FECV type II are antigenically closer to TGEV or CCV than to FIPV type I viruses [54, 113].

FIPV UCD 1, FIPV UCD2 and FIPV-Black are type I viruses which produce slow CPE in cell-culture and are mainly cell-associated. Type II includes the virulent FIPV-79-1146 which grows rapidly in many cell lines, and FECV-79-1683 [31,113].

Antibodies against FECV neutralise FIPV in vitro [114]. However, some MAbs have been developed which recognise different isolates of FIPV and FECV by different epitopes in the spike [29, 30, 31, 54]. The peplomer of a FECV isolate was very similar to that of a virulent FIPV isolate suggesting that M, N or some non-structural protein or any combination may form the basis of pathogenicity in FCoV [31].

It is possible that FECV and FIPV represent pathogenetic variants of a single FCoV type which possesses a broad spectrum of virulence from asymptomatic infection through enteritis to lethal disseminated FCoVV [9, 114]. TGEV, porcine respiratory CoV, CCV and FCoV may be regarded as host range mutants, or biotypes, rather than different 'species' [7, 9, 11, 58, 95, 106, 122, 123] because of their considerable antigenic cross-reactivity.

Feline anti-FIP VNA neutralize CCV [82, 122, 164] and some, but not all, neutralize TGEV [26, 58, 119, 122, 153, 156, 164]. Canine antisera against CCV neutralize FIP poorly [86, 164] but strongly neutralize TGEV, and antisera against TGEV neutralize FIP and CCV [164]. Hence between FIP and CCV there is a one-way cross-reactivity. Titres by IF and VN are higher with homologous virus, so that the 3 viruses can be distinguished serologically [122].

By immunoblotting, N and M of TGEV, CCV and FIPV were recognised by sera against all three viruses despite the different sizes of M (M_{TGEV} was 29K, M_{FIPV} was 24K, M_{CCV} was 22K or 32K). Anti-CCV sera precipitated only trace amounts of FIPV N. Results for S had been unobtainable by immunoblotting. However, by ELISA S of all 3 viruses was recognised by

heterologous sera [58].

A cloned cDNA probe for TGEV hybridized with CCV and FIPV but not H 229E [82, 130]. It may be that the major epitopes responsible for immunological cross-reactivity of various strains of FIPV, FECV, TGEV and CCV are quite discrete from sites mediating virulence. Minute deviations in molecular sites governing virulence may produce major differences in tropism for mononuclear phagocytes, perhaps influencing both humoral sensitisation as well as ability of pathogenic strains to replicate within these target cells [15, 21, 72, 138].

1.2.3.1 TGEV causes vomiting and diarrhoea in piglets under one month of age though older animals can be affected. TGEV mainly replicates in the epithelium covering the tips of the villi of the small intestine (SI) and can cause stunting and fusion of the villi [32, 122]. Virus is shed from the GI tract for as long as 8 weeks p.i. [84]. A porcine coronavirus which grows primarily in the respiratory tract and cross-reacts with TGEV has been identified [32]. This virus differs from TGEV by only a few epitopes on the spike [17].

The FCoV nucleotide sequence is 79% identical to that of TGEV. The amino acid sequence of the M protein has overall homology of 84.7%, and the N protein has 82.2% homology [151].

TGEV can infect cats causing seroconversion and virus shedding for up to 3 weeks but no clinical signs are apparent [11, 26, 56, 57, 122, 126, 127, 139]. Exposure to TGEV does not usually sensitise cats to FCoV [9].

FCoV readily infects the intestinal mucosa of newborn pigs where it causes lesions almost indistinguishable from those seen in mild TGEV infection [9, 110, 113, 122].

1.2.3.2 CCV was first isolated in 1971 from faeces of dogs suffering from gastrointestinal disorders [58]. It can infect

cats causing seroconversion and is shed from the oropharynx for at least a week [9, 11, 82, 126, 127, 139]. Reports conflict as to whether CCV sensitises cats to FCoV infection [83] or not [9, 138] although different isolates were involved. However, effusive FCoVV resulted after i/m and i/p inoculation of a British strain of CCV [82, 83].

A modified live CCV vaccine inoculated intramuscularly resulted in clinical signs in dogs similar to FCoVV [27, 114].

1.2.3.3 H 229E causes common cold in human beings [84]. In cats this virus causes seroconversion and asymptomatic infection but not antibody dependent enhancement (ADE) of FCoV infection [9, 11, 126, 127]. ADE is discussed in 1.4.7.

1.2.3.4 Coronavirus-like particles (CVLP) have been observed in the faeces of cats. They are larger than FCoVs, 90-300nm in diameter, with spikes which are 25 nm long and have spherical or tear-drop like knobs attached to the particles by thin stalks [61]. These viruses have not been grown in culture. CVLPs do not elicit antibodies cross-reactive with FCoV or cause disease in cats [61, 103, 113, 136]. They may still be excreted 12 months post infection [61,136].

1.2.4 Growth of FCoV in cell culture

The growth of FCoV in cell culture has been notoriously difficult to achieve [45, 61, 100, 102, 110, 111, 141]. Primary virus isolation may need as many as 5 blind passages before CPE can be detected [164]. Even where the technique was successful, virus could only be isolated from the tissues of about 1 in 7 field cases of FCoVV [114]. Recovery of cell culture-adapted FCoV from experimentally infected cases is also difficult [141, 143].

Initially, virus was passaged in laboratory animals: in cats [111, 158], suckling mice, rats and hamsters [57, 100, 104, 106]. The first cell culture in which FCoV was found to

replicate was in cells of peritoneal exudate of experimentally infected kittens which were mainly macrophages [100, 104, 106, 110, 137, 139]. FCoV has also been grown in feline small intestinal organ cultures [60, 62, 104, 110], tracheal ring cultures [104, 110], feline embryo lung, [14, 93, 110, 140] foetal cat head and intestinal culture [164], FEA [140], Crandell feline kidney (CRFK) cells [27, 39, 85, 86] (in which FIPV is said to form larger plaques than FECV) [86, 146], and feline catus whole foetus (fcwf) cells [15, 29, 39, 113, 114]. Fcwf cells can phagocytose latex and carbon particles, have Fc receptors and stain positive for aspecific esterases and therefore are identified as macrophage-like cells [73].

One-step growth of FCoV has been reported [132]. In foetal feline cells the production of virus begins at 6 hours p.i. and reaches a maximum at 12 hours p.i. Fifty to 99% of the infectivity remains cell-associated [20, 73]. Other authors have found peak virus production at 30-48 hours p.i. in FEL cells [14, 86, 140]. In macrophages, virus is first detected in the supernatant 8 hrs p.i. peaking at 2 days p.i. Virus production continues for 4 days then declines until 8-10 days. Each macrophage produces 10-100 infectious virus particles but no CPE is apparent [137].

As early as 6.5 hours p.i. the first cytopathological changes (rounding and swelling of cells) can be observed. At 8 hours p.i. the cells have formed syncytia [20]. CPE is similar in FEL [140], CRFK [67, 140], A-72 [140] and FEA cells [140]. Cell fusion and syncytium formation are mediated by the FCoV spike [22, 110]. In fcwf cells, viral antigen can be detected on the cell surface by IF at 16 hours p.i. when CPE is most pronounced [73]. Multinucleated giant cells were observed 3 days p.i. in a CRFK culture inoculated with viruses isolated from 2 field cases and there was complete cell destruction by 5 days p.i. [85, 113]. Primary foci enlarge from the peripheries and secondary foci are seen only occasionally, indicating that infection proceeds mainly by cell to cell contact. As the foci

grow, cells in the centre slough into the media [113].

FCoV is assembled at the membranes of the smooth endoplasmic reticulum (ER). Virions do not bud from the plasma membrane [14, 31] but intracellular budding of virions takes place between the rough ER and the cis-Golgi [14, 45, 57, 62, 148, 152]. The virions acquire their lipid envelope from the cells, excluding host cell proteins in the process [132]. Matrix protein accumulates in the Golgi and is believed to determine the site of virus budding. Mature virions are released from the cell by exocytosis [131, 148].

A proportion of the S is not incorporated into virions and is transported to the plasma membrane where it induces cell-to-cell fusion [31, 148]. Conflicting reports exist as to whether spike can be detected on cell surface by IF [22, 148].

1.3 Epidemiology

1.3.1 Geographical and species distribution

FCoVV has a worldwide distribution [56, 60, 104, 105, 106].

Although primarily a disease of domestic cats FCoVV has been recognized in lions, mountain lions, leopards, jaguars, lynx, caracal, sand cat and pallas cat [57, 104, 105, 106]. The cheetah is reported to be particularly susceptible [16, 27, 91].

From time to time a FCoVV-like disease occurs in other species of animals. To date none of these conditions has been shown to be associated with FCoV [126].

1.3.2 Genetic predisposition to FCoVV

The development of FCoVV is likely to depend on several factors, one of which is genetic predisposition [9, 138].

In a worldwide survey, no difference was found in the

prevalence of antibody between domestic and pedigree cats [56]. Similarly, in a series of necropsies at the University of California no apparent breed predisposition was found [102]. However, taking the figures for pedigree cats as a whole in that survey, the prevalence was 14.3% compared to 9.8% in domestic cats.

In a survey of the prevalence of FCoV antibodies in the USA, a higher mean titre was found in Persians and Himalayans [12]. In the UK, of 100 cases of FCoVV 72 were in pedigree cats and 28 were in domestic cats. A predisposition amongst Burmese was noted [140]. The more frequent appearance of FCoVV in pedigree than domestic cats may not be due to genetic predisposition, but rather to the cattery environment in which these cats are usually reared [104, 106].

An incident which favours the argument for genetic predisposition is that in 1982/3 90% of a cheetah colony succumbed to FCoVV. High FCoVV mortality has been attributed to the genetic uniformity of individuals in this species leading to genetic susceptibility [91]. This may be correlated with the intrinsic susceptibility of the cheetah macrophage to infection [139]. However, variation of the number of FCoV infected macrophages in in vitro infection did not depend on the individual cat from which the macrophages were obtained [137]. Alternatively, the monomorphism of the major histocompatibility complex (MHC) of cheetahs might be responsible for a poor immune response to viral epitopes involved in the induction of protective immunity [91].

Resistance of mice to MHV is genetically controlled. The expression of genetic resistance is manifested at the level of the mature macrophage and since these cells are involved in primary viral replication, their interaction with virus strongly determines the outcome of infection [132, 139]. It has been proposed that FCoV virulence is determined by antigenic differences between isolates in the peplomer protein

which mediates binding of virus to cellular receptors [30, 31]. However, attenuated FCoV strains ceased to replicate in macrophages 3-4 days p.i. compared to virulent strains which were still producing infectious virus 10-12 days p.i. This finding suggests that additional critical steps in CoV infection of macrophages occur after the virus has adsorbed and gained entry to the cell. The avirulent and virulent FCoV strains grew to comparable titres in CRFK cells, therefore it was concluded that the macrophages are intrinsically more resistant to avirulent than virulent strains of FCoV [139].

Other evidence in favour of genetic predisposition is that several siblings in a litter and repeated litters from the same queen may succumb to FCoVV and then the queen may herself contract the disease [27, 98, 104, 106].

1.3.3 Age

FCoVV has been recognised in cats of all ages from stillborn and newborn to aged animals over 14 years old [105, 106]. However, it is more usually a condition of younger cats [8, 104]. In one cattery which lost 59 cats the average age was 5.8 months (range 2-11 months) [116]. In an American survey of cats which had died of FCoVV, 41 out of 153 were less than a year old; 47 were 1-2 years old; 24 were 2-3 years old; 20 were 3-4 years old [101]. In the UK, 51 of 100 FCoVV cases were under a year old and 16 were 1-2 years old [140]. In both surveys, the prevalence declined after 4 years of age and thereafter there was only sporadic occurrence of FCoVV.

In a survey of the presence of antibodies in cats, an inverse relationship was noted between age and mean FCoV antibody titre [12].

1.3.4 Sex

In some early reports males were found to be affected slightly more frequently than females [105, 116]. FCoVV [140] and FCoV antibodies [56] were more common in male cats than female but

the difference was not significant when compared to the sex incidence in the general population. In another report, no association was found between sex and the presence of antibodies or disease [12] and it is currently generally accepted that there is no significant sex predisposition [104, 106].

1.3.5 Concurrent FeLV/FIV infection

The immune component of FCoVV might lead one to speculate that concurrent infection with FeLV or FIV, which suppress the immune response, would affect the outcome of infection with FCoV [114]. In two experimental colonies with no history of FCoVV, immune suppression by FeLV and methylprednisolone [104, 114] or cyclosporin A [76] caused outbreaks of FCoVV.

Early reports stated that about 50% of all cases of FCoVV had concurrent FeLV infection [43, 101, 153]. In a more recent survey the frequency of FCoVV cases which were FeLV positive was 19.3% and FCoVV was the third most common disease condition associated with FeLV [117]. However, the prevalence of FeLV infection in the total necropsy cat population was 16% in that survey, so the frequency was not significantly above average [118].

In the UK, 3% of cases of FCoVV were found to be FeLV positive [140]. In another report, FCoVV was cited as the major cause of death in FeLV infected cats and no difference in incidence of FCoVV was noted between FeLV infected cats and those with concurrent FIV and FeLV infection [129].

In Japan no association was found between the incidence of FCoV antibodies and FIV infection. FCoVV was less common among FIV infected cats than would be expected in the population as a whole [69].

1.3.6 Prevalence

In ordinary cats from pet households, the prevalence of FCoV antibodies is estimated to be between 10 and 40% of the population [94, 95, 102, 103, 109, 110, 111, 114, 153, 155].

In catteries antibodies are either completely absent or occur in 80-90% of the cats [9, 12, 57, 94, 102, 103, 109, 110, 111, 153, 155].

In most cases, the occurrence of FCoVV is sporadic and the disease can apparently disappear from a household of cats and then reappear months or years later [106]. Although the infection rate is high among cats, less than 5% of cats infected with FCoV develop FCoVV [102, 104, 109]. Much higher rates have been seen in some isolated catteries. For example, a 3-49% yearly loss in kittens was observed in 1 cattery over a 4 year period in an area where the cats were kept together in large numbers. By contrast, in another part of the same cattery where the cats were isolated from each other FCoVV mortality was very low (3 of 8000 cats) [116].

1.4 Pathogenesis

1.4.1 Virus transmission and source

The route by which FCoV is spread in nature is still unknown [114] but it is likely that initial infection is probably oro-faecal or oral-oral [9, 48, 49, 111, 113, 140] and/or by inhalation of virus into the trachea [9, 111, 141].

FCoV is a highly infectious virus infecting over 90% of cats in any cattery in which it is endemic. Sick cats cannot be the only source of virus because FCoVV is sporadic in occurrence and commonly many months or years pass between the episodic appearance of cases of disease within catteries [111]. Besides, in experimental infections, by the time cats are clinically ill, they are no longer shedding virus [140, 141].

The virus does not survive for long periods outside the host [100], and indirect transmission on litter trays [111] and clothing [9, 103] has been shown to occur, however, environmental virus is not thought likely to be a major source of infection [64].

Transplacental transmission is possible but so far is unproven [9, 98, 99, 101]. The best evidence for this source was the isolation of FCoV from a 4 day old kitten [85, 114] and the recognition of FCoVV in stillborn and weak newborn kittens born to a queen that had FCoVV during the latter stages of pregnancy [104].

An additional source of FIPV might be cats infected with FECV. Mutation of the latter into the former has been postulated [104, 106, 108, 114].

Most instances of virus transmission are probably direct [8] and the most likely source of FCoV is healthy carrier cats [104, 111, 114, 141]. It has been suggested that after transient URT signs, most cats recover and may become persistently infected and shedders of virus [126]. The existence of healthy carriers has been demonstrated by placing tracer kittens with healthy seropositive cats [103, 111, 141]. Cats with higher antibody titres appeared to be more infectious than cats with lower titres because the kittens seroconverted in a shorter time [103, 111]. Latent FCoV infection was demonstrated by infecting healthy FIPV immune cats with FeLV and dosing with methylprednisolone: 6 out of 8 died of FCoVV [114, 115]. This condition occurred 0-4 months after FCoV exposure but not 7-9 months later, suggesting that either virus is lost with time from the body, or that immunity is strengthened [107].

Whether transmission by haematophagous arthropods occurs is unknown [9] but is considered to be unlikely [116].

Experimentally, FCoV has been administered i/v [87, 101], i/p [49, 87, 101, 109, 114], s/c [101], orally [43, 49, 114], intratracheally [153], intranasally [114], and by aerosol [153, 158]. In one experiment i/p inoculation of NW1-FIPV produced seroconversion and effusive FCoVV in 100% of kittens, intratracheal inoculation of the same dose caused FCoVV in 6 of 10 kittens and seroconversion in one other, and oral inoculation of caused disease in 3 of 15 kittens and seroconversion of another 1 [110]. Route of infection obviously has an important role in the outcome of the infection.

Abdominal exudate, organ extracts, whole blood, and urine from naturally infected cats have been used to transmit infection [43] though urine was not found to be infectious in one study [101].

1.4.2 Virus shedding

FCoV is probably excreted into the environment in oral and respiratory secretions, faeces and possibly urine [8, 9, 26, 48, 114, 153]. It is probably not shed in large amounts by seemingly healthy cats [112]. Cats with persistently high FCoV antibody titres probably shed more virus than do cats with lower titres [8, 111].

In experimental infections of cats which had been infected i/v or i/p, virus was detected in the oropharynx on the second day p.i. and continued to be excreted for 9-10 days p.i. Cessation of virus shedding seems to be associated with development of systemic IgG and both circulating and salivary IgA. A second short episode of virus shedding occurred around day 14 p.i., about the time of onset of clinical signs. One cat in each of 2 experiments did not show this later shedding episode and did not go on to develop FCoVV. One possible reason for the difficulty in isolation of virus from field cases may be that most cats stop shedding before any clinical signs develop [82, 141, 142, 143].

In one experiment virus shedding in faeces began 2-7 days p.i. [48, 141] and continued to 10-14 days p.i. There was no secondary episode and an SPF cat which was placed in contact with a recovered experimental cat for 2 years failed to seroconvert [141].

FCoV has been detected in the faeces of naturally occurring cases of FCoVV [48] and FECV has been detected in the faeces of carrier cats [103, 111].

1.4.3 Virus dose

Increasing virus dose was shown to increase the proportion of cats infected with virulent NW1-UCD1-FIPV or avirulent FIPV-Black strains. Some cats which were given a small dose of virus became immune while others, given the same dose, failed to become infected or else became infected and died. The authors concluded that cats in the field are exposed infrequently to relatively small amounts of virus [112].

Seronegative cats given low doses of FCoV developed a relatively higher incidence of non-effusive FCoVV, while higher doses caused predominantly effusive FCoVV [104].

1.4.4 Primary sites of virus replication

The sites of primary virus replication are probably the epithelial cells of the small intestine [42, 50, 104, 110, 113, 126, 143, 160], possibly the tonsils [113, 143] and the oropharynx [110, 126].

FECV selectively infects the apical columnar epithelium of the intestinal villi from the caudal part of the duodenum to the caecum [111]. In a naturally occurring case, FECV antigen was observed only in the epithelial cells of the caecum but no other tissue [111]. FECV also replicates to a lesser extent in the tonsils and mesenteric lymph nodes [113]. It is claimed that the essential difference between FIPV and FECV is that the latter does not spread any further than the intestinal

epithelium and regional lymph nodes [104].

In experimental infections of cats, FCoV (FIPV) was detected by fluorescence in the jejunal enterocytes 12 hours p.i. By day 5 the whole SI was infected and infection persisted even to 12 days p.i. [48, 51]. The Wellcome strain of FIPV was detected in the caecum 2-14 days p.i. and in the lumen of the colon 3-14 days p.i. [143]. One author reported that in most cases antigen-containing cells in the intestine decreased from day 7-8 p.i. along with regeneration of the mucous membrane and onset of antibody production [48], while another showed that antigen was most obvious at 7-14 days p.i. [143]. In naturally occurring cases, viral antigen can be detected in epithelium of the duodenum, jejunum, caecum and colon even in the presence of high titred serum antibody [48].

In intestinal ring organ cultures, the UCD and Dahlberg strains of FIPV replicated in the cytoplasm of columnar epithelial cells of villi [48, 62]. The changes observed were similar to, though less severe than, those seen in infections by CoVs of other species which produce diarrhoea. In the absorptive epithelial cells of the SI, microvilli were shortened, blunt and decreased in number. Mitochondria assumed abnormal size and morphology, the endoplasmic reticulum dilated and there were increased lipid droplets in the cytoplasm. FCoV secretion continued from day 1-43 in these cultures [62].

In a small proportion of cats infected with FCoV, the virus leaves the gut and becomes systemic. Two explanations have been offered for this progression. First, in these cats mucosal immunity is insufficient to prevent the virus leaving the gut; and secondly virulent FCoVs are more able to infect monocytic phagocytes in vivo so that viral replication is not restricted to the intestinal epithelium [111, 139]. In these cats it is believed that the primary target cell for viral replication is the macrophage [49, 51, 73, 88, 100, 109, 113, 137, 139, 158]. Viral particles have been observed by EM in

cytoplasmic vacuoles and SER of degenerated macrophages in inflammatory lesions of experimentally infected cats [104, 158] and in the monocytic cells in the medullary sinuses of the mesenteric lymph nodes [111]. In natural infections, CoV particles can sometimes be detected by EM in peritoneal macrophages and mesothelial lining cells [45]. FCoVV could be transmitted using white blood cells from infected cats but not plasma or white blood cell free erythrocyte suspensions [157]. In mice, the primary site of coronavirus replication of mouse hepatitis virus is in the macrophage. [132].

How the virus leaves the gut is still a matter of speculation but may be via macrophages [19, 160] draining to the mesenteric lymph node or, in seropositive cats, via neutrophils which take up antigen-antibody complexes before infection of enterocytes or macrophages [51]. The virus is then transmitted throughout the body in the monocytes [126] or neutrophils [51] and enters the perivascular area of affected organs after the monocytes attach to and migrate through the walls of the vessels. The resulting perivasculitis is the initial lesion observed in histopathological sections of clinical FCoVV [51,126].

In naturally occurring cases and orally infected experimental cats, FIPV was detected by immunofluorescence (IF) in mesothelial cells, macrophages of the omentum and serosa of abdominal organs and spleen [45, 48]. Additionally, after i/p infection, FCoV antigen was found by IF in lesions in the spleen, liver and mesenteric lymph nodes [49]. At 5-12 days p.i. some macrophages in the lamina propria of the S.I. and sinuses of the mesenteric lymph nodes showed virus-specific fluorescence [48]. In an experimental infection FCoV was detected in Peyer's patches 7-14 days p.i., and slight fluorescence was found around the portal areas of the liver 14 days p.i. Virus was not detected in any other organ [143].

Fluorescent antibody staining of tissue sections from cats with both forms of disease demonstrated the presence of FCoV in the

lesions. In effusive FCoVV, a large amount of viral antigen was contained in the phagocytic cells that make up the periphery of the pyogranulomatas, in Kuppfer cells within and adjacent to hepatic sinusoids and in macrophages within parenchymatous lesions in the liver. There was less viral antigen in the lesions of non-effusive FCoVV and it was usually found within a few macrophages adjacent to vessels in the centre of the lesions [106].

Intrinsic resistance of feline peritoneal macrophages determines whether or not they will support FCoV replication. They are more resistant to avirulent laboratory strains than virulent strains which grow to comparable titres in CRFK cells [137, 139]. In vitro, macrophages produce virus for at least 6-8 days. Virus infection of macrophages is non-cytolytic which may indicate that FCoV-infected macrophages are important for persistence of infection and the carrier state in vivo [137].

Secretion of IFN by epithelial cells and macrophages may be important early host factors in containment of primary mucosal infections. During viral infections, endogenous beta-IFN is generally released into the circulation within hours after initial viral replication. This circulating IFN and also gamma-IFN from migrating virus-sensitised lymphocytes can protect distant target organs from virus invasion. In vitro, treatment of feline cells with homologous beta-IFN or human alpha-IFN prevented or reduced subsequent viral replication in cultures challenge exposed to FCoV [160].

In kittens infected by aerosol, FCoV viraemia occurred 2 days p.i. in seropositive kittens and 6 days p.i. in seronegative kittens and lasted at least 7 days in the former (sampling was stopped at 9 days p.i.), 13 days in the latter [157, 158]. In these kittens, initial localisation and replication of virus occured in large mononuclear cells in regional lymphoreticular tissues and/or subepithelial tissues of the URT. This early viral replication was associated histologically with

hyperplasia of reticuloendothelial and lymphoid cells in T-cell dependent paracortical areas of regional lymph nodes [158].

Haematogenous spread of virus or virus-infected cells after local infection in lymphoreticular tissues may subsequently result in infection of sinusoidal macrophages and reticuloendothelial cells of target organs that contain extensive blood sinusoids (i.e. liver and spleen). Infection of specialised macrophages (i.e. pericytes) in the tunica adventitia of blood vessel walls or diapedesis of infected leukocytes from the circulation might also result in early vascular and perivascular infection in systemic tissues [158].

1.4.5 Nature of the antibody response to FCoV

Spontaneous cases of FCoVV usually have markedly elevated serum anti-FCoV antibody titres [101, 156, 157].

Most cats infected with FCoV, whether diseased or healthy, produce VN and IF antibodies [114]. There is no correlation between the presence of fluorescent or VN antibodies and either the development of disease or immunity [112,114]. VN antibodies in the sera of field and experimental cases can have titres of over 40,000 but have no protective value [57, 82]. IF and VN neutralising antibodies appear at the same time [112] but there is no correlation between the titres of each test. Antibodies detected by IF are probably against soluble antigens within infected cells and their persistence is often correlated with the persistence of the eliciting agent [112].

VN antibodies are believed to recognise the virus spike [31, 112, 148]. These antibodies often persist for some time after the virus is eliminated. In kittens IF antibody titres peaked rapidly then dropped to undetectable levels in a month or so, whereas VN antibody titres remained high [112].

VN antibodies may initially be IgM, because in a measurement of class-specific response, VN activity was detected 5-7 days p.i., as

was the IgM response. The IgG response was not noted until 5 days later [82].

VN antibodies prevent virus entering cells in vitro. However, two virulent isolates of FIPV and one isolate of FECV were able to spread from cell to cell in culture despite the presence of neutralising antibodies in the medium [31].

IF antibodies recognise M [59, 65, 163]. The initial response of experimentally infected cats was to the M protein of the virus as measured by competitive ELISA (CELISA) [163]. Anti-N antibodies appeared 60 days p.i. while there was no measurable anti-S response. Of 205 field samples that were seropositive by CELISA, 98.5% had anti-M antibody, 58.5% had anti-N antibody and 4.8% had anti-S antibody. Only cats that developed antibodies to S had VN antibodies, although some succumbed to infection [163].

Cats which have confirmed FCoVV had antibodies to a 95K protein which may be a monomeric form of the spike [67].

IgM, IgG and IgA were produced to the same level against all viral proteins [82].

Antiviral antibodies may not be totally responsible for the hypergammaglobulinaemia seen in FCoVV [59, 68, 155]. IL-6, which is produced by peritoneal exudate cells in cases of FCoVV, regulates terminal differentiation of B cells into antibody secreting cells [38, 52] and appears to be involved in the polyclonal B cell activation in several diseases such as rheumatoid arthritis [38]. Another theory is that viral antigen can non-specifically stimulate B cell proliferation in the absence of T helper cells. If some B cell clones happen to be autoreactive, autoantibodies will be produced [41].

1.4.6 Maternally derived antibody

Upon nursing, kittens acquire FCoV antibodies from the queen. Levels of these antibodies decline during the first weeks of life with a $T_{1/2}$ of 7 days, and by 4-6 weeks titres are generally less than 16. In many kittens, antibody reappears in the serum around 8-14 weeks of age as the kittens become themselves infected while in others it remains at a low level [107, 111, 114].

IgG in colostrum and IgA in milk is protective because kittens are not usually infected until between the fifth and tenth weeks of life [103, 116]. In TGEV infection, immune pigs confer immunity on their piglets through their colostrum and milk [84]. Kittens born to FCoV immune mothers have a solid immunity to FCoV on challenge between 8 and 12 weeks of age [107, 114]. Whether this was due to MDA or inability to mount an antibody response due to their youth was unknown. However, if not infected until 22 weeks of age some kittens will be immune and others develop enhanced disease [107,114].

1.4.7 Antibody dependent enhancement

Antibody dependent enhancement (ADE) is the name given to the phenomenon whereby cats with either naturally or experimentally acquired serum anti-FCoV antibodies experience a more rapid fulminating FCoVV [9, 49, 82, 95, 109, 110, 111, 114, 139, 141, 154, 156, 157, 158] and/or enteritis [51] following FCoV exposure than do FCoV antibody negative cats receiving the same challenge dose. Seronegative kittens passively treated with immune serum or purified IgG develop disease with the same frequency, acuteness and severity as seropositive kittens [109, 156]. This phenomenon has foiled many vaccine attempts with homologous virus [11, 138], CCV [82, 83, 138], TGEV [11], or recombinant vaccinia virus expressing the S protein [149].

The mechanism by which ADE occurs is poorly understood. It has been likened to dengue haemorrhagic fever (DHF) in man [109, 139, 156] in which cases of disease occur as secondary

infections in patients with pre-existing serum antibodies to dengue viruses. It is postulated that upon reinfection with a heterologous serotype of dengue virus, the patient rapidly develops high titres of cross-reacting but non-neutralizing antibodies. The formation of large amounts of virus-antibody complexes leads to activation of complement and haemorrhagic shock syndrome [156]. Heterotypic immunity to different strains of FCoV may be involved in the pathogenesis of FCoVV [113].

Several possible mechanisms of ADE have been considered. Non-neutralising, opsonising antibodies may accelerate uptake of FCoV into target cell monocytes or macrophages [9, 49, 104, 113, 156, 158]. The exact mechanism is not understood, but it is assumed that the F_c portions of the immunoglobulins bind to the mononuclear cells promoting attachment and uptake of virus [126]. Enhanced infection of feline macrophages in vitro by virulent FIPV isolates occurred when the viruses were incubated with anti-FCoV antibody before inoculation [137, 139].

Thymectomized kittens had more severe lesions and more prominent FCoV antigen positive macrophages than sham-operated kittens infected in the same way. This result suggested that partial depletion of T cells due to thymectomy enhanced viral growth or spread to macrophages [9, 50].

Surface expression of FCoV antigens on macrophages seen late in infection when virus progeny are already formed, could induce antibody-mediated lysis of infected cells and therefore promote increased dissemination of virus particles. This could explain the decrease in complement levels in terminal FCoVV cases. A pronounced increase in T lymphocytes is also seen terminally in FCoVV cases which may be an indication of enhanced direct lymphocyte-mediated cytotoxicity [73].

A third mechanism might be promotion of widespread vasculitis through immune complex (IC) deposition [9, 49, 158]. Antibody

reacts with antigen and complement possibly resulting in a local Arthus-like response [109, 113].

However, the mere presence of FCoV antibody in the serum of a cat does not mean that FCoVV will ever develop in that cat, even after repeated FCoV exposure. FCoVV is a relatively uncommon condition in nature and the majority of FCoV antibody positive cats will never develop the disease [9].

1.4.8 Timing of the antibody response

At 3 days p.i. no antibody response could be detected by IF, ELISA or VN. By 5-6 days p.i. VNA were detected and by 9 days antibodies were detected by IF or ELISA [66, 82].

In experimental infections with FCoV, antibody was seen in the serum 4-14 days p.i. [103, 114, 141] and continued to rise until death VN titres were higher and appeared sooner than IF antibodies [141]. Onset of clinical signs coincides closely with the appearance of antibodies in the serum [156].

In an experiment which examined the class specific response, IgM was produced 3-7 days p.i., peaked at 7-14 days, then decreased in cats which succumbed to FCoVV. In one cat which survived, the IgM response remained high. IgG production began 7-12 days p.i. IgA response began 7-12 days p.i., peaked at 12-17 days then either remained constant (in a cat which died of FCoVV) or decreased to background levels (in a survivor). [82].

In natural infections, SPF kittens placed with healthy FCoV carriers seroconverted without developing fever, in 2-10 weeks [111, 114] and 2-3 weeks after exposure to an infected litter tray [111].

1.4.9 Immune complexes

There is a convincing case that FCoVV is an immune complex-mediated (IC) disease. Circulating ICs [70, 71, 82, 88, 139,

141, 156] and glomerular IC deposition [71, 88, 109, 156] have been demonstrated. Complexes of IgG and C_3 bound specifically to FCoV were demonstrated in the cytoplasm of infected macrophages [109, 158] and in lesions by IF [109, 156]. Pre-existing antibody enhances the course of the disease and seronegative cats survive longer than seropositive cats [51, 88, 109, 112, 156, 158]. The C_3 component of serum complement activity decreases several days before death [51, 71, 109, 141, 156]. Passive antibody titres decrease more rapidly in FCoV-challenged kittens compared with unchallenged controls suggesting that antibodies are actively consumed during the course of disease in addition to spontaneous degradation [156].

Antibody reacting with circulating soluble antigen forms immune complexes the size, valency and solubility of which depend on ratios and amounts of antigen and antibody and on the size of the antigen. Such complexes are trapped in small blood vessels [25].

The nature of the antigen, ratio of antigen:antibody, class and subclass of antibody and interaction with the complement system and cellular elements such as macrophages, polymorphonuclear leukocytes play important roles in the fate and biologic activity of the IC [25].

Monovalent antigens such as haptens do not form lattices, therefore ICs remain in circulation for long periods without tissue deposition. However, multivalent antigens such as some proteins do combine with antibody to form lattices and ICs of varying composition depending on molar ratio of the reactants [25].

In large antigen excess small ICs are formed which do not fix complement. In antibody excess the ICs can fix complement but are rapidly removed from the circulation by the lymphoreticular system [25, 121, 158]. Pathogenic ICs capable of initiating inflammation when antigen excess is modest, form ICs

intermediate in size and solubility and are capable of fixing complement but are not rapidly eliminated from the circulation by the reticuloendothelial system [25].

An Arthus-type reaction is a localised lesion of venules that results when antigen-antibody complexes, formed during periods of antibody excess, deposit in venules and fix complement. Furthermore, ICs formed in antibody excess are more effective activators of complement than are complexes formed at equivalence or antigen excess [158]. In FCoVV, as in Aleutian disease in mink, ICs form in antibody excess because cats with FCoVV usually have high FCoV serum antibody titres [156, 158].

The pathological consequencess of ICs are dependent on their ability to activate the complement system [25]. If seropositive infected cats are decomplemented using cobra venom factor FCoVV does not ensue [71]. Complement is probably activated by the classical route in FCoVV (the alternative route is unlikely because C3 and C4 levels are depleted simultaneously) [71].

Following deposition of the IC in the blood vessel wall, the Fc portion of IgG fixes C3a [88]. C3a has chemotactic properties attracting neutrophils which invade the vessel walls [25, 57, 158]. C3a also has anaphylactic properties capable of triggering vasoactive amine release from platelets, mast cells and their circulating counterpart, the basophil [71, 120]. The vasoactive amines released include histamine and 5-hydroxytryptamine [120] which cause endothelial cell retraction and thus increased vascular permeability [120, 156, 158]. Contraction of capillary endothelial cells allows exudation of plasma proteins. Neutrophils pass through the gaps between the endothelial cells and release lysosomal enzymes, particularly collagenase and elastase, causing necrosis of the vessel wall. [25, 57]. The neutrophil is the characteristic cell species encountered in FCoVV granulomas [57, 71].

ICs also interact with platelets through their Fc receptors

causing aggregation and microthrombus formation [158] and release of more vasoactive amines. Several cases of disseminated intravascular coagulation (DIC) have been induced in FCoV antibody positive kittens experimentally infected with FCoV [113, 156]. Platelets will also clump at sites of endothelial injury [156]. Alternatively, there may be accelerated (antibody-mediated?) lysis of platelets in sensitised kittens [156].

Immune complex deposition is most likely where there is high blood pressure and turbulence. In glomerular capillaries blood pressure is four times that of any other capillaries. Vessel bifurcations also cause turbulence and filters such as the choroid plexus or ciliary body of the eye are likely sites for IC deposition [121]. FCoVV lesions are commonest in the peritoneum, kidney and uvea, all sites of high blood pressure and turbulence.

In effusive and enhanced FCoVV, the clinical and pathological signs are attributable to the vascular damage resulting from acute IC disease. The damage to the blood vessel walls allows the protein-rich plasma to pass into the peritoneal or pleural cavities resulting in the characteristic protein-rich fluid of high specific gravity seen in effusive FCoVV [126, 156].

1.4.10 Immunity

(See also 1.4.6)

The development of FCoVV depends on two events: an intrinsic susceptibility of the alimentary or respiratory tract epithelium to infection and an immune response that is non-protective. In one experiment, all cats challenged by the i/p route, 6/10 challenged intratracheally and 3/15 challenged orally became infected [110]. The majority of cats exposed to FCoV by natural routes are resistant to infection [111, 116].

If primary infection of FCoV were limited to the URT or GIT, local immunity might inhibit systemic spread of virus [85, 153].

There are several reasons for believing that once FCoV has left the GIT, recovery is cell-mediated [27, 88, 159].

1. The humoral immune response is not protective. Transfer of hyperimmune serum from an immune individual to a susceptible cat results in enhanced disease in the recipient [104, 112, 113, 114, 141].

2. Non-effusive FCoVV resembles tuberculosis and deep mycotic infections and the immunity to these infections involves mainly cellular mechanisms [104, 114].

3. The incidence of FCoVV can be increased by concurrent infection with FeLV. This effect may be due to suppression of cell mediated immunity and T cell mediated humoral immunity by FeLV [114]. FCoV reached peritoneal macrophages more commonly in thymectomized kittens compared with control kittens where it was more commonly found in enterocytes suggesting that T cells have a role in local protection against FCoV. It has been suggested that FCoV impairs the function of T cells [50]. Thus, following inoculation with FCoV the response of T cells to the mitogen concanavalin A was profoundly reduced and only recovered to pre-inoculation levels in cats which survived infection [141].

4. Healthy carrier cats carry FCoV as a latent or sequestered infection which can be reactivated by infection with FeLV. A carrier state of this type is common in microbial infections where cell mediated immunity (CMI) is known to be the primary protective mechanism [114]. Immunity may depend on viral persistence in the body and be lost when virus is lost [107].

5. In aerosol infection with FCoV early viral replication was

associated histologically with hyperplasia of the reticuloendothelial and lymphoid cells in T-dependent areas of regional lymph nodes. Proliferation of mononuclear cells in these areas is consistent with early stimulation of CMI [158].

6. Transfer of T cells from suckling heterozygous mice (which are less susceptible to FIPV) to nude mice (which are very susceptible to FIPV) protected the latter [145].

Experimentally, it has been difficult to demonstrate CMI. In one experiment only 4 of 8 immune cats showed delayed-type hypersensitivity reactions when FCoV antigen was injected into the third eyelid [114]. Weiss and Cox demonstrated strong delayed-type hypersensitivity response in the skin of a cat which was immune to FCoV and none in a cat which succumbed [159]. Only 2 cats which survived longest and 4 of 17 in another experiment showed a specific lymphocyte blastogenesis to FCoV antigen [114, 141].

Persistent IgM production may protect cats from developing FCoVV. A suggested mechanism is that IgM competes with IgG for virus, since IgM does not opsonise antigen for macrophage uptake, less virus would be phagocytosed and therefore be able to replicate in macrophages [82].

1.5 Clinical signs

The majority of FCoV infections are asymptomatic. Most cats naturally exposed to FCoV develop antibodies without showing clinical signs and less than 5% of infected animals succumb to the disease [57, 102, 105, 106, 111, 155, 156].

Pedersen has suggested that the form of FCoVV which follows viral dissemination depends on the type of immunity which develops: humoral immunity and strong CMI can lead to recovery or latent infection [113]. If cats with latent infection become immunosuppressed, reactivation of the virus occurs and effusive or non-effusive FCoVV ensues. Cats with humoral

immunity and partial CMI develop non-effusive FCoVV and cats with humoral immune response and no CMI develop effusive FCoVV [104, 113].

1.5.1 Feline enteric coronavirus

FECV causes mild to moderately severe enteritis lasting 2-5 days [126] in SPF kittens 5-12 weeks old [103, 110, 111, 114]. It is rarely fatal [96, 103, 114]. In SPF kittens 12 weeks of age FECV infection causes mild to inapparent signs and adult cats show no signs of infection [96, 103].

Pyrexia occurs 3-10 days p.i. and lasts 2-5 days. Diarrhoea and vomiting occur just prior to fever. Vomiting precedes diarrhoea by 12-48 hours. The kitten becomes anorexic and dull during and after the diarrhoea and there may be mucus and blood in the faeces. Diarrhoea lasts 48-96 hours. Some kittens become dehydrated [103, 111, 113].

In an adolescent cat with naturally occurring disease there was mesenteric lymphadenopathy, gross oedema of the bowel and mucus-laden diarrhoea [103].

Cats experimentally infected with FECV which eliminated the virus were observed for 3-24 months and did not develop FCoVV [103, 111].

Cats inoculated i/p with a strain of FECV remained afebrile and became seropositive 7 days p.i. However, by the third passage of this virus in vivo one of two kittens became very ill and febrile and virus was found in the mesenteric lymph node [114]. Cats inoculated with the Bristol strain of FECV developed FCoVV [33].

1.5.2 Clinical signs of FCoVV

In most cases (over 75% [153]) the initial infection is asymptomatic or signs are subclinical [9, 11, 102, 106, 114, 126, 153]. The primary signs of FCoV infection tend to be mild

enteric [27, 28, 85, 110, 126, 140] or upper respiratory signs characterised by slight ocular and or nasal discharge which persists for 1-4 weeks and occurs weeks to years before the onset of clinical signs of FCoVV [85, 110, 126, 153, 156, 157]. These signs occur throughout an affected cattery and not only in those cats which develop FCoVV [140].

The clinical signs common to almost all cases of FCoVV are chronic, fluctuating pyrexia [8, 28, 29, 42, 57, 95, 97, 104, 106, 116, 126], anorexia [8, 27, 28, 42, 95, 97, 104, 106, 116, 126], weakness or listlessness [28, 29, 42, 57, 95, 97, 104], and weight loss [27, 29, 42, 57, 71, 95, 104, 106, 116, 126]. Some cats are bright and with good appetite in the early stages of clinical signs [78, 104].

In experimental infections pyrexia occurred at the same time as the appearance of antibodies, 7-10 days p.i. when virus was inoculated i/p [109, 110, 156] and 8-20 days after intratracheal inoculation. Following oral infection fever occurred 20-75 days after the first dose of virus and FCoVV occurred 2-6 weeks later [109, 110]. Antibody levels increased throughout the course of the disease [110, 111]. Seropositive kittens develop fever 24-48 hours p.i. [109, 156].

Abdominal distension may be present [27, 29, 104, 106, 156] due to ascites [28, 57, 95, 97, 104, 126]. Pleuritis occurs in about 40% of effusive cases leading to pleural effusions [106, 126] and dyspnoea [57, 104, 106].

Diarrhoea has been reported in cases of FCoVV [97]. FCoV was recovered from a 1.5 year old cat with fatal enteritis [85] and diarrhoea was observed intermittently over a period of 10 months in cheetahs which went on to die of FCoVV [27]. Certain FIPV isolates causing only diarrhoea have been reported [27, 48].

In experimental intragastric infections, diarrhoea with blood

was sometimes seen 2-3 days p.i. [140]. Certain strains of FIPV are capable of producing FCoVV or enteritis or both [9, 48, 49, 50, 51, 67]. Vomiting sometimes occurs [77, 85, 97].

There is usually no pain on abdominal palpation [57, 97]. The mesenteric lymph nodes may be palpable [126] and the kidneys may feel irregular [104].

Clinical signs in dry FCoVV depend on which organs are affected. Lesions are found in the peritoneal cavity of 50% of cats with non-effusive FCoVV and in the pleural cavity of 10%. Unlike effusive FCoVV, there is a high incidence (33%) of ocular and CNS signs [106].

Ocular signs are common [57, 126] and include uveitis [97, 104, 106, 114]; precipitation of cellular debris in the internal face of the cornea [97] causing punctate corneal opacities [28, 104] (in a case of wet FCoVV); hyphema [28, 104]; one pupil constricted more than the other [28]; haemorrhage on retina [28]; sheathing of the retinal blood vessels [104]; chorioretinitis [106] and nystagmus [78, 106].

CNS and cerebellar-vestibular signs have been reported in 35% of non-effusive and 10% of effusive cases [8, 95, 126]. These include convulsive disorders [88, 104, 106]; inco-ordination [78, 104, 106, 111, 116]; ataxia [116]; posterior paresis [8, 104, 106, 116]; head tilt [8, 88, 106]; circling [88, 106]; hyperaesthesia [104, 106, 116]; dementia, personality changes (rage, withdrawal) [88, 104, 106]; and brachial, trigeminal, facial and sciatic nerve palsies [104]. Hydrocephalus secondary to disease of choroid and ependyma has also been reported [8, 78, 88, 104, 106].

Jaundice [95, 97, 104, 111, 116]; dehydration [97, 116]; orchitis [95, 104, 116]; and rough hair coat [126] have also been attributed to FCoVV.

Six cases of colonic granulomatous FCoVV were reported which presented as weight loss, constipation, vomiting and palpable abdominal masses, later shown to be colon. Contrast radiographs showed narrowing of the lumen of the intestine in the descending colon [147].

In cheetahs, erosions around the nostrils and lips, and depigmented areas above the incisors as a result of ulcerative glossitis were also reported [27].

1.5.3 Prognosis

In natural infections of the effusive kind, death ensues approximately 1-6 weeks after the onset of clinical signs [29, 97, 106, 116] but can occur up to and sometimes beyond three months [28, 87, 95]. In non-effusive FCoVV, the course is 1-12 weeks but can be more protracted on occasion [106].

In experimental infections death is often much more rapid, characteristically 15-20 days p.i. [42, 109] especially if the cats are seropositive, when clinical signs can appear 36-48 hours p.i. and death may ensue in 5-7 days [109].

In the literature, there are a few accounts of experimentally infected cats which developed clinical signs and recovered [42, 114].

1.6 Pathology

1.6.1 Gross pathology

There is no clear-cut distinction between effusive and non-effusive forms of FCoVV. Parenchymal lesions can occur in effusive FCoVV and a small quantity of fluid can occur in non-effusive FCoVV [57, 95]. For example, a laboratory kitten which showed CNS signs had fibrinous plaques on the surface of its spleen and liver indicating that the cat had undergone an earlier bout of peritonitis [111].

The characteristic lesions of effusive FCoVV are 0.5-3mm grey-white foci containing fibrin. Lesions may be found on the visceral and parietal peritoneum, pleura, pericardium, mesentery, omentum, on the diaphragm, and within lungs, thymus, liver, germinal follicles of spleen, kidneys, pancreas, and abdominal and thoracic lymph nodes [42, 45, 49, 51, 57, 104, 116, 156, 158].

The characteristic lesion of non-effusive FCoVV usually consists of irregular solitary or multiple granulomas, which are most commonly within the kidneys, but may occur in any organ [104, 106, 116].

Ascites

The ascites in effusive FCoVV is variously described as clear, yellowish, light amber or straw coloured [9, 57, 104, 106, 116, 126, 143, 156] and in quantities varying from a few ml [158] to 500 ml in experimental cases [143] and less than 100ml to 2,500ml in natural cases [42, 97, 116]. It is viscous [106] and characteristically clots on exposure to air [42, 57, 97, 116, 128, 143]. Up to 20 mls of similar fluid may occur in the thoracic cavity [116, 143] in 20% of cases [97].

There may be fibrinous adhesions between organs [95, 105]. The omentum is often thickened and oedematous [45, 106, 116].

Alimentary tract

In experimental infections with FIPV, the SI and LI showed oedematous thickening of either anaemic or hyperaemic mucosa with watery or mucous content [48, 49]. In FECV, there was gross oedema of the bowel [103]. The colon and sometimes terminal ileum and caecum can be thickened, firm and fibrotic due to granulomatous lesions of FCoVV. This condition can occur with or without FCoVV involvement of other organs [147].

Kidney

In the non-effusive form, lesions occur most frequently in the

kidneys and consist of grey-white raised nodules 2mm-5cm in diameter, or opaque streaks scattered over the cortical surface and extending into the cortex. In advanced cases, the nodules become confluent and replace large segments of the renal parenchyma [28, 57, 87, 106, 116].

Brain

Grossly, lesions in the brain and spinal cord may be virtually absent in cats which show CNS signs. When found, lesions may be small (1-5mm) yellow-gray nodules and plaques most commonly on the ependyma and choroid plexus of the lateral and fourth ventricles, meninges and ventral surface of the spinal cord [78, 87, 116]. The aqueduct and fourth ventricle may be filled with thick, grey, opaque material. Slight roughening and brownish discoloration approximately a mm deep of the ventricular surfaces may occur [8, 78]. Hydrocephalus with dilatation of the lateral ventricles, cerebral aqueduct and fourth ventricle and thin cerebral cortex has been reported [78, 116].

1.6.2 Histopathology

The characteristic lesion of FCoVV is a necrotising phlebitis or perivasculitis of smaller veins, arteries and lymphatic vessels [45, 57, 87, 109, 126, 151, 155, 156]. The lesion is characterised by a central focus of necrosis surrounded by a perivascular infiltration of mononuclear cells, proliferating macrophages and lymphocytes, plasma cells and neutrophils [28, 45, 49, 156, 158]. Endothelial and subintimal mesenchymal proliferation was also reported in a field case [28]. Fibrin and protein-rich fluid are deposited within and around the lesions [106]. Many of the vessels contain fibrinous thrombi [87, 156].

The lesions of non-effusive FCoVV are more granulomatous in nature. The outer zone is more fibrous and the numbers of plasma cells, lymphocytes and histiocytes are much greater. Oedema, hyperaemia and fibrin and protein exudation are not as

pronounced as in effusive FCoVV [78, 87, 106, 113].

Serosae

The surface of the omentum, mesentery, peritoneum, abdominal organs and sometimes the pleura are covered with a greyish-white fibrinous deposit with a diffuse or focal infiltration of lymphocytes, plasma cells, histiocytes, macrophages, neutrophils and cellular debris [27, 42, 45, 97, 116, 143]. Sometimes the inflammatory lesion extends into underlying tissues along penetrating blood vessels [106].

In naturally occurring FCoVV, EM of omentum and cells spun out of ascitic fluid can occasionally reveal CoV particles in the peritoneal macrophages and mesothelial lining cells [45].

Kidney

In the kidney, there is subcapsular distribution of nodular lesions and their orientation is around superficial veins [155]. IgG and C_3 precipitated in the glomeruli cause nephritis [47, 49, 70, 71].

Alimentary tract

The enteric lesions of FIPV are degeneration, desquamation and hyperplasia of the epithelial cells and oedematous tunica propria with some infiltration of neutrophils and mononuclear cells. Fusion of the epithelial cells is frequent. In a few cases, the deep mucosa and tunica muscularis of the SI and LI had remarkable cellular infiltration of lymphocytes, plasma cells and neutrophils [27, 48, 51]. The villi of the SI are shortened and fused to one another [48, 143]. Peyer's patches are atrophied [48]. In the colonic form, granulomatous lesions originate in the deep layers of the colon, perhaps the serosa, and extend transmurally [147]. In natural infections of cheetahs, superficial gastric erosion was found in some animals [27].

In FECV infection, the target tissue is the mature apical

columnar epithelium of the intestinal villi from the mid-duodenum to terminal ileum and caecum. Fusion of adjacent epithelial cells in the tips of the villi, fusion of the villi, sloughing of the tips of the villi and villous atrophy occur in severe cases [103, 111].

Lymphoid tissue

In experimental oropharyngeal infection, the mandibular and mesenteric lymph nodes show diffuse and focal expansion of lymphoid tissue, congestion, oedema and infiltration by inflammatory cells [143]. In experimental aerosol infection of seronegative kittens, the initial paracortical hyperplasia is followed by follicular hyperplasia and formation of germinal centres which may indicate a specific primary humoral immune response. In seropositive kittens, lymphoid depletion and severe necrosis develops rapidly in regional lymph nodes [158]. Follicular hyperplasia [147, 156] or depletion [156] have been noted by other authors. In natural infections, gross lymph node involvement is usually limited to the thoracic and abdominal lymph nodes [106].

The mesenteric lymph nodes showed sinus catarrh with accumulation of lymphocytes, plasma cells, polymorphonuclear neutrophils, cellular debris and histiocytes as well as nodular hyperplasia of macrophages, some of which contained erythrocytes [42, 48, 51, 87, 97, 156, 158]. In non-effusive FCoVV there may be nodular lesions in the mesenteric lymph nodes. Peyer's patches may be hypertrophied [28].

Splenic enlargement may be due to histiocytic and plasmacytic infiltration of the red pulp [51, 106], hyperplasia of the lymphoid elements of the white pulp [28, 87, 106, 156], necrotising splenitis with fibrin deposition and neutrophil cell infiltrates or by a more organised pyogranulomatous reaction [106, 156]. Other accounts found the follicles of the lymph nodes and spleen were atrophied [27, 48].

Slight neutrophil cell infiltration of tonsils occurred 1-14 days p.i. Neutrophils and macrophages were present in the tonsils and epithelium and in the surface exudate of inflammatory cells and fibrin. By the time the cats showed clinical signs, there were no longer histological changes present in the tonsil, though there were still changes in the mandibular lymph node [48, 143].

Eye

Lesions in the eyes [42] consist of focal to diffuse collections of macrophages, neutrophils and fibrin in the iris, ciliary body, ciliary processes and anterior chamber [8, 87, 116, 158]. Oedema and small aggregates of cells were present in the retina. The anterior chamber may contain clumps of cells, some adhering to the corneal endothelium and forming keratic precipitates [28, 116]. Corneal oedema may occur [116]. Focal and diffuse pyogranulomatous inflammation of meninges and optic nerve have been recorded [8, 87].

Brain

As many as 63% of spontaneous cases of FCoVV have neuropathological lesions though fewer present with neurological signs [8].

The pattern of inflammation of the brain characteristic for FCoVV is also seen in the encephalitides of equine infectious anaemia and visna in sheep. Since all three disorders are believed to be immunologically-mediated, their common patterns probably reflect zones of the CNS prone to IC and/or cell mediated injury [8]. Another possible cause of neurological signs in FCoVV is ischaemic encephalopathy secondary to the vasculitis [88].

In the choroid plexus, ependyma of the fourth ventricle and meninges of the brain stem, leptomeninges, eighth cranial nerve, medulla oblongata, cerebellar peduncles and cerebellar white matter and pons there may be perivascular cuffing by

mononuclear cells, neutrophils, fibrin and necrotic debris [8, 28, 42, 78, 155, 158]. Reactive astrocytes with enlarged vesicular nuclei occur under the region of mononuclear cell infiltration [8].

The fourth ventricle contains eosinophilic, proteinaceous exudate with a few macrophages, neutrophils, lymphocytes, plasma cells and cellular debris. Blockage of the normal flow of CSF may result in secondary hydrocephalus [8, 78, 88].

Bone marrow

Bone marrow showed an increased number of megakaryocytes and immature myeloid elements but decreased erythropoeitic cells [28, 85, 87].

1.7 Biochemistry

Biochemical tests are useful ancillary tools in the diagnosis of FCoVV. The most useful parameter is the serum albumin:globulin (A:G) ratio which is usually low in cases of FCoVV (<0.6) [28, 42, 155].

CSF and aqueous humour in cats with CNS signs show a similar increase in protein as in the serum, unless the disease is localised in the subependymal area [78, 88, 104, 106, 116, 155]. Creatinine phosphokinase does not normally cross the blood-brain barrier; so its presence in the CSF reflects either vascular leakage or destruction of CNS tissue, in one case of FCoVV it was 46 SU/ml, (normal is less than 1 SU/ml) [78].

Urine may be changed in cases of FCoVV. For example, protein may be present, depending on urinary tract lesions. However these changes are not consistent enough to aid diagnosis [28, 79, 104, 116].

1.7.1 Serum

Measurement of serum proteins in FCoVV is a useful adjunct to diagnosis. However caution must be advised in interpreting serum proteins of the cat because there is a wide variation in normal values [42] depending on age, sex, diet, stress and method of analysis. With age, albumin levels decrease whereas globulin concentration increases [92].

In FCoVV total proteins are raised to over 78 g/l in 55% of effusive cases and 70% non-effusive [8, 57, 78, 79, 95, 96, 104, 106, 126, 155, 156]. This is caused by a variable increase in gamma-1 (IgG1) gamma-2 (IgG2) [9, 155] alpha-2, beta and gamma globulins [8, 42, 104, 106, 155] but not IgA or IgM [42]. (Alpha-2 globulins are fibrinogen or haptoglobin and beta-1 globulin is transferrin). Haptoglobin increases in intravascular haemolysis, non-specific injury and inflammation and is raised in the serum of cats with FCoVV [44].

A polyclonal hypergammaglobulinaemia is frequently reported in clinical cases of FCoVV [12, 28, 42, 79, 95, 97, 116, 156] with globulin levels of over 46g/l [155]. It is not known if this polyclonal gammopathy is specific for FCoV [57, 155].

In experimental infections, gamma globulins showed a smooth polyclonal increase over the period of testing [142].

Cats with experimental FCoV infection exhibit a biphasic acute phase protein response. Orosomucoid, haptoglobin and a beta-2 protein which may be transferrin rose slightly the day after inoculation with FIPV then showed a large negative response before gradually returning to normal levels terminally. Two other bands in the alpha-2 region showed a similar biphasic response, these corresponded to ceruloplasmin and alpha-2 macroglobulin of humans, although these proteins are not yet identified in the cat [142].

These acute phase proteins are not associated with immune

complex deposition. However, in experimental FCoVV C_3 shows a small rise initially, followed by a drop which rose again about 7 days before death, when there was a further fall [71] which mirrors the acute phase protein response [142].

Acute phase proteins are largely induced by the action of macrophage-derived cytokines (IL-1, TNF, possibly IFNs) on hepatocytes and the profiles seen may result from the involvement of the macrophage in the pathogenesis of FCoVV. The initial increase may result from cytokine release by virus-infected cells and the negative response may be due to depletion of functional phagocytic cells as demonstrated by suppression of clearance of ^{125}I-labelled polyvinyl pyrollidone in FCoV infected cats. The terminal rise may also be associated with inflammation seen in later stages of disease or DIC [142]. However, it has been shown that FIPV triggers release of IL-1 from peritoneal exudate cells [36, 40] and alveolar macrophages [37]. Alternatively, the cytokine involved may be IL-6 which induces positive and negative phase protein responses [52]. IL-6 is found in the sera and ascites of cats with naturally occurring FCoVV but not in healthy cat sera [38].

It has been suggested that a biphasic acute phase protein response is unique to FCoV infection but it has no practical application because changes occur before significant clinical signs appear and a surviving cat showed a similar response to those which succumbed to FCoVV [142].

Bleeding time, prothrombin time and partial thromboplastin times are all increased where there is DIC [81, 104, 106, 154, 155]. Also, depression of coagulation factors 7, 8, 9, 11 and 12, elevated fibrin-fibrinogen degradation products, and thrombocytopaenia are associated with DIC in infected cats [154, 155]. Serum fibrinogen is raised [81, 95, 104] in 45% of cases [155]. Fibrinogen rises as early as 8 days p.i. in serum and plasma [42].

In experimental infections C3 levels became elevated, reaching peak values 7 days before death, then decreased as levels of circulating ICs and anti-FCoV antibodies increased [71, 95, 156].

Liver damage due to FCoVV lesions may result in raised liver enzymes, serum glutamic-pyruvic transaminase; serum glutamic-oxaloacetic transaminase and sorbitol dehydrogenase and in raised bilirubin [57, 81, 95, 104, 126, 155, 156].

Renal damage may result in raised blood urea or creatinine [28, 81, 95, 155].

Lesions may involve the pancreas causing a pancreatitis, increased serum lipase and possibly amylase. Diabetes mellitus secondary to FCoVV has been reported [155].

1.7.2 Ascites

The ascites in cases of FCoVV is an exudate, not a transudate. It is light amber or straw coloured [57, 106, 126] and often contains fibrin tags [42, 57, 95]. The coagulation on exposure to air operates in 2 stages: the first is fibrinogen to fibrin and the second is slower, independent of the action of the usual anticoagulants and depends on factors destroyed after a period of 16 hours at 56^{o}C. Differences in the extent of coagulation of ascites depends on the temperature at which it is stored: those frozen to -20^{o}C were totally coagulated, those stored at 4^{o}C contained a large clot, at 20-37^{o}C there were small clots and at 56^{o}C ascites was cloudy without coagulation [42].

Proteins in ascites are raised though less [42] or more [116] in quantity than those of serum. Protein measurement of pleural or peritoneal effusions from cats is useful in establishing or ruling out a diagnosis of FCoVV. Effusions from a series of 12 cases of FCoVV were compared with 47

effusions due to other diseases. If gamma globulins represented over 32% of total protein then the animal was likely to be suffering from FCoVV. If gamma globulins were less than 32%, or if albumin was over 48%, or the A:G ratio was over 0.81, then the animal was unlikely to have FCoVV [128].

Ascites or pleural effusions from FCoVV cases typically have high total protein, in the range 45-80g/l [28, 57, 79, 81, 95, 104, 106, 116, 128, 155] due to its high gamma globulin content [28, 79, 95, 116]. This property (along with cytology and serology) serves to distinguish FCoVV from CCF, hepatic disease or neoplasia [79] though does not help to distinguish it from lymphocytic cholangitis.

IL-6 is produced by peritoneal exudate cells in naturally occurring cases of FCoVV. More IL-6 is found in ascites than sera. No IL-6 was found in healthy cat sera. Ascitic IL-6 was inversely correlated to serum albumin:globulin ratio [38]. IL-6 regulates the acute phase response of the liver [38, 52]. IL-6 in ascites may leak into the systemic circulation and be linked to systemic alterations such as pyrexia, enhanced synthesis of immunoglobulin and acute phase proteins [38].

Ascites in cases of FCoVV has high specific gravity (1.017-1.047g/l) [9, 28, 57, 81, 95, 155] and is always sterile [28, 79, 155].

Haptoglobin values are less in ascites than in serum and therefore are not useful in diagnosis [44].

1.8 Cytology

1.8.1 Haematology

Haematological changes are variable and not diagnostic of FCoVV in themselves [155]. Leucocytosis is the most commonly reported change [42, 57, 78, 79, 81, 95, 96, 97, 104, 106, 116, 155] and occurs shortly after the onset of fever [42].

Occasionally, a leukopaenia has been reported [57, 79] especially in experimental cats [155]. In kittens experimentally infected with a strain of FECV white blood cell counts dropped to less than 50% of normal at the onset of fever and diarrhoea, due to a fall in neutrophil count [103, 111]. The neutropaenia persisted for the period that diarrhoea was present.

Neutrophilia [42, 81, 85, 97, 104, 106, 116, 155] of up to 90% [42, 79] or over [97] occurs, with a shift to the left [57, 78, 79, 155]. Inclusion bodies have been seen in the circulating neutrophils of some cats with FCoVV which may be immune complexes [106] or Dohle bodies [28].

Lymphopaenia [42, 81, 85, 96, 97, 104, 106, 116, 155] and eosinopaenia [42, 57, 97] may occur.

Red blood cell counts are variable. Mild anaemia may occur [28, 42, 79, 81, 97, 106, 116, 155] and is normocytic and normochromic [57, 95]. Alternatively, the red blood cell count may remain unchanged [42, 79]. Thrombocytopaenia is associated with DIC [154, 155, 156].

1.8.2 Ascites

Ascites contains a high number of wbc (1,600 - 25,000 wbc per microlitre) [104, 106] consisting of neutrophils [79, 96, 116, 155], lymphocytes [116], macrophages, plasma cells, fibroblasts [79] and mesothelial cells [28, 106, 155]. It may also contain erythrocytes [28]. Cats in the acute stage of FCoVV have a high ratio of neutrophils to mononuclear cells, whereas more chronic cases have increased numbers of macrophages, lymphocytes, plasma cells and mesothelial cells [155]. Cytology of abdominal or thoracic fluids shows an inflammatory disease process which can be useful in eliminating differential diagnoses of abdominal tumour [24].

1.8.3 CSF

CSF and aqueous humour in cats that show CNS signs or ocular signs contain 90-9250 cells, mostly neutrophils, per microlitre [78, 88, 104, 106, 116, 155].

1.9 Diagnosis

Definitive diagnosis of FCoVV can only be made by histopathology of a biopsy or post mortem sample [9, 78, 126, 155]. Most clinical diagnoses at present rely on evaluation of history, presenting signs and results of serology, biochemical and haematological tests [9].

FCoV itself is notoriously difficult to isolate from field cases [26, 96, 114].

1.9.1 Indirect immunofluorescent assay

FCoV-infected cell culture or tissue sections from cats with FCoVV are reacted with different dilutions of test serum or ascites. After incubation and washing, an anti-feline IgG conjugated to fluorescein isothiocyanate (FITC) is applied [5, 10, 26, 66, 67, 102, 114, 141, 155, 157]. Tissue sections used are commonly liver [80, 102, 109] or FCoV infected suckling mouse brain [50].

Occasionally TGEV [5, 26, 56, 57, 66, 94, 155] or CCV [27, 85, 155] is used instead of FCoV. Antibody titres obtained from heterologous IFA may be lower than with homologous virus [26, 66, 94]. Reactions with H229E are generally weak and not acceptable for diagnostic purposes [5].

IF has also been used to locate viral antigen in situ [106, 156, 158] using rabbit anti-FIPV or canine anti-CCV sera [85].

1.9.2 ELISA

Purified FIPV, FECV, or TGEV is attached to a 96 well plate, the serum to be tested is added, then anti-cat IgG conjugated

to an enzyme is added to each well [66, 67, 82].

Kinetics-based ELISA (KELA) differs from conventional ELISA by giving values which can be converted to an antibody titre [12].

A competition ELISA for the detection of immune complexes has recently been developed by SmithKline Beecham but is reported not to compare well with IF [124].

1.9.3 Virus neutralization

VN is determined by a plaque reduction test. Dilutions of test sera are mixed with virus suspension for one hour at 37°C then are inoculated onto confluent cell culture. FCoV is usually used but TGEV may be employed [94, 119]. An agar overlay is applied after a period of adsorption and the plates are incubated until plaque formation occurs. Titres are expressed as the serum dilution which inhibits a proportion of plaques [4, 66, 85, 141, 164]. Titres as high as over 40,000 have been reported in field cases [57].

VN may be more sensitive than IFA or ELISA [82] for detection of an early response to FCoV infection. However, 17/89 serum samples positive by ELISA and IF for anti-FCoV antibodies were negative by VN. Most of the antibody activity of these samples was directed against M and N rather than S [66].

1.9.4 Uses of serological tests

There are several ways in which serological tests are useful in FCoV infection.

1. As an aid to diagnosis: presence of anti-FCoV antibodies will not diagnose FCoVV, but a negative test will usually rule it out [9, 12, 104, 153, 155]. Cats with FCoVV generally have high IFA antibody titres, even over 25,000 [8, 9, 12, 26, 27, 57, 67, 94, 102, 106, 153, 155, 158]. Cats with effusive FCoVV had titres of 1:400-1:25,600, cats with non-effusive FCoVV had titres of 1:1,600-1:25,600 [102]. It has been claimed that

titres of over 1:1600 are uncommon in cats infected with FECV but common in FIP [104, 111]. However, cats with FIP have been reported to have lower titres [134].

2. Screening of catteries for exposure to FCoV [9, 153, 155].

3. Screening new cats for entry into seronegative catteries [9, 153]. Cats should be isolated and retested 2 weeks later [153].

4. Prognosis: serology can be used to monitor exposure to FCoV and a prognosis can be made depending on how elevated the antibody titre becomes [27]. A precipitous rise in titre may mean a poor prognosis for the cat [153]. The antibody levels of 2 cats with non-effusive FCoVV monitored over a 14 week period rose 30-100 fold [102]. However, there may be a decrease in antibody titre, especially in the terminal stages of disease [126]. Prolonged elevated titre suggests possible viral persistence in carrier animals [12] though some animals which go on to die have persistently high titres for a long time before developing illness.

5. Monitoring treatment [155].

1.9.5 Limitations of serological tests

It should be appreciated that there are several limitations in the use of serological tests.

1. The inability to distinguish virulent, vasculitis-causing infections from asymptomatic or enteritis-causing FCoVs, CCV or TGEV infection [9, 11, 23, 66, 82, 96, 104, 105, 111, 126, 133]. Antibody to TGE could be detected by IF only after parenteral hyperimmunisation [56]. Cross-reactivity with HCV 229E is not a problem [11].

2. The presence of antibodies in both diseased and healthy animals renders diagnosis of FIP and prediction of prognosis

difficult [3, 7, 9, 11, 12, 80, 95, 96, 101, 103, 105, 106, 133, 153, 155].

3. FCoVV cases may have low antibody or no detectable antibody [9, 104, 126].

Five explanations have been offered for this:

a. the cat is moribund. Antibody sometimes disappears from the circulation during the terminal stages of disease [9, 94, 102]
b. extensive immune complexing means there is little free, unbound antibody available to be detected [9, 126]
c. peracute disease process where there is a sluggish antibody response (e.g. in young kittens) which is harder to detect, especially if the test uses heterologous virus [9, 94, 155]
d. wrong diagnosis [155]
e. more than one serotype of FCoV [126]. In examining feline field sera against FCoV and CCV, those cats which were positive against one isolate and negative against another were the cats with lower titres [82].

4. Cats with other diseases which may appear like a manifestation of FCoVV may coincidentally have FCoV antibodies [9, 11, 155].

5. The presence of antibody does not indicate immune status because the cats which develop FCoVV are also seropositive [9].

6. There is a lack of correlation between presence of antibodies with virus excretion though it is possible that cats with lower antibody titres excrete less virus than those with higher titres [110, 153]. The tests should never be used in a test and removal programme [9].

7. Non-specific reactions: cats may develop antibody against bovine serum components that may be present in vaccine preparations. Bovine serum is used in cell cultures as a

nutrient for growing both vaccine virus and the antigen used in serological tests [6, 9, 10, 12, 29, 126]. As many as 6% of all sera tested in one laboratory had increased background [10, 12]. This reactivity usually lasts up to 3 months [6, 10, 12] but can be persistent in some cats with no history of recent vaccination. This finding suggests that persistent antigen stimulates antibody-producing cells, perhaps by frequent comsumption of bovine products, milk or beef [10].

1.10 Treatment

By the time clinical signs appear, treatment is generally of little value and the course of the disease is almost invariably fatal [104, 116, 126, 138, 155]. Treatment is aimed at modifying the immune response. Use of immunosuppressive and antineoplastic drugs such as cyclophosphamide, phenylalanine mustard and azathioprine have produced only temporary remission and have had unpleasant side effects [104, 153]. Large doses of corticosteroids, usually prednisolone, are the treatment of choice [116, 126, 138, 155]. Recently, studies of an immunomodulating drug, Promodulin, indicated 40% of cats treated showed remission of anorexia, pyrexia and serosal effusion [162].

Antibiotics for control of secondary infection are also given. Although certain anti-viral agents such as human alpha-IFN and feline beta-IFN have been shown, in vitro, to inhibit replication of FCoV, they would only be of use during early virus exposure [160].

Transfusion of immune sera fails to protect and in fact enhances disease [112].

In cats which are showing CNS signs, the clinician must remember that many immunosuppressive agents do not cross the blood brain barrier. If seizures are present, cats can be treated with 1.1mg/kg/day of phenobarbital divided into 2 doses

[88].

It has been reported that cats with only ocular signs may recover with local corticosteroid treatment and that antibodies to FCoV will eventually decrease [104, 126].

1.11 Vaccine stategies

To be truly effective, a vaccine must evoke long-lasting CMI, therefore it is unlikely that a killed virus vaccine would work. The ideal vaccine would be a modified live product that will persist long enough to evoke CMI [107, 114].

One problem is strain variation. Cats that appear solidly immune to one or more strain of FCoV can develop FCoVV when inoculated with another strain [114]. Protective immunity may not be inducible in some cats [112, 113].

1.11.1 Homologous virus

Some cats can be immunised using sublethal doses of virulent FCoV. However, where some cats became immune, others given the same dose by the same route would develop FCoVV. Furthermore, the same dose of virus that would either kill or immunise some cats failed to infect others [19, 112, 113]. When FECV was used to inoculate cats, there was some protection in some cats but enhanced disease in others [110, 111]. Obviously these results are too inconsistent to advocate the use of virulent virus or FECV as a vaccine [19, 112, 113].

There are various reports of unsuccessful vaccine attempts using FCoV strains:

Cats inoculated with FIPV-UCD2 were resistant to reinfection with the homologous virus but were susceptible to infection with FIPV-UCD1 and FIPV-79-1146 and showed ADE [114].

FIPV-UCD3 given oronasally evokes solid immunity without causing FCoVV. The problem is that the cats become immune

carriers of the virus and may develop disease if their immunity is depressed [114].

Cats inoculated with low virulent strains will either develop FCoVV or seroconvert without illness. If the latter group is challenged with more virulent virus, some will be immune and some develop accelerated effusive FCoVV [107].

Cats inoculated with avirulent strains oronasally produced IF and VN antibodies. They were not protected against oronasal challenge and some developed enhanced disease. There may be a factor present in virulent virus that is absent in avirulent virus but is necessary for protection [112].

1.11.2 Heterologous virus vaccines

Attempts to vaccinate cats with TGEV failed to protect against subsequent challenge with FCoV [19, 26, 66, 112, 113, 138, 139].

Vaccination with an unattenuated field isolate of CCV by aerosol or s/c failed to protect or enhance [19, 138]. In another experiment, CCV caused enhancement to FCoV and further inoculation induced pathological lesions characteristic of effusive FCoVV [82].

Attempts to vaccinate cats with HCV 229E caused an homologous antibody response but neither protection nor sensitisation was observed [11, 19].

1.11.3 Recombinant vaccinia-FCoV vaccine

Recombinant vaccinia viruses expressing S, N, and M have been used to vaccinate cats. The spike-expressing recombinant evoked enhanced disease. Immunisation with the N protein recombinant had no apparent effect on the outcome of challenge. Three of 8 kittens immunised with the M protein recombinant survived challenge with virulent virus compared with one of 8 control kittens [149, 151] suggesting that an anti-M response

is important in recovery.

1.11.4 Temperature-sensitive mutant vaccine

The most hopeful vaccine candidate to date is a temperature-sensitive mutant of FCoV which replicates at 31^{o}C but not at 39^{o}C. This mutant could be recovered from the URT, cervical and mandibular lymph nodes and tonsil, but from nowhere else in the cat. Mucosal immunity may be important in stopping the primary infection of FCoV [19, 34, 35].

CHAPTER 2

MATERIALS AND METHODS

MATERIALS

2.1 Media

Dulbecco's Minimal Essential Medium (MEM) was purchased from Gibco and was supplemented with 200mM L-glutamine, 100mM sodium pyruvate and 3.75% sodium bicarbonate. Foetal bovine serum (FBS) was used at 10% for cell growth and 1% for virus growth.

For storage of cells in liquid nitrogen, 10% FBS and 10% dimethyl sulphoxide (DMSO) were added to the above.

2.2 Cell culture

Feline embryonic fibroblast cells of the FEA line [74] were used between passages 20-45. The cells were routinely grown in 250ml plastic flasks (Nunclon) and 2.5l glass roller bottles. The cells were routinely sub-cultured 1:4 twice a week.

2.3 Feline coronavirus (FCoV)

The Wellcome strain of FIP virus was used [93]. Virus was cloned three times by picking plaques under agar and stock was prepared as described in 2.2.2.

Table 2.1 Stock solutions and buffers for SDS-PAGE

Acrylamide stock	
acrylamide	30%
bis-acrylamide	0.8%
made up in distilled water (DW).	
Ammonium persulphate (AMPS)	
10% in DW.	
Coomassie blue staining solution	
Coomassie Blue	0.05%
methanol	30%
glacial acetic acid	10%
Destaining solution	
acetic acid glacial	7%
made up in DW	
Reducing sample buffer (RSB)	
tris/HCl pH 6.8	0.0625M
SDS	2%
2-mercaptoethanol	5%
glycerol	10%
Bromophenol blue in DW	1%
In non-reducing sample buffer the 2-mercaptoethanol was omitted.	
SDS-PAGE running buffer	
glycine	192mM
tris base pH 8.6	25mM
SDS	0.1%
SDS-PAGE running gel	
acrylamide	10%
bis-acrylamide	0.33%
Tris pH 8.6	0.4M
SDS	0.1%
TEMED	0.07%
DW	
AMPS	0.025%
SDS-PAGE stacking gel	
acrylamide	5%
bis-acrylamide	0.17%
Tris pH 6.8	0.12M
SDS	0.1%
TEMED	0.07%
DW	
AMPS	0.075%

Table 2.2 Stock solutions and buffers for immunoblots

Alkaline Phosphatase (AP) buffer	
NaCl	100mM
$MgCl_2$	5mM
diethanolamine pH 9.5	100mM
BCIP	
BCIP (disodium salt)	5%
dimethylformamide	100%
EDTA	
EDTA	500mM
in DW	
Immunoblotting buffer	
glycine	192mM
tris base pH 8.6	25mM
SDS	0.01%
methanol	20%
NBT	
NBT	5%
dimethylformamide	70%
PBS/Tween	
Tween 20	0.05%
in phosphate buffered saline (PBS)	
Solution A	
non-fat milk powder	1%
in TBS	
Tris-buffered saline (TBS)	
NaCl	144mM
tris	25mM
pH 8.0 with HCl	

2.4 Cell growth and sub-culture

Cells were grown in 25 cm^3, 80 cm^3 or 175 cm^3 plastic flasks or 2.5 litre glass roller bottles. To make sub-cultures of FEA cells growing in monolayers, the old medium was removed and the monolayer was washed with 10 or 20 ml trysin-versene prepared by adding 20ml trypsin-EDTA (Gibco) to 180ml PBS. The washing was repeated and the flask or roller bottle was incubated at 37°C for 5 minutes. Then the detached cells were suspended in medium, were carefully dispersed and one quarter of the cells was seeded in 25 or 100ml medium. A mixture of 5% CO_2 in air was added and the culture was incubated at 37°C. Sub-cultures were made twice a week.

2.5 Preparation of FCoV

Confluent FEA monolayers were infected with FCoV at a ratio of 10 pfu FCoV/cell. After 2 hours at 37°C the inoculum was removed and 5 or 20ml medium containing 1% FBS was added. The virus was harvested 24 hours later, spun at 10K for 10 mins and frozen to -70°C. Stock prepared in this way had a titre of 2 x 10^7 pfu/ml. Before using to inoculate new cells it was filtered using a 450nm Micropore filter (Flow).

2.6 Assay of FCoV

Six-well plates (35mm diameter) were seeded with 5 x 10^5 FEA per well. Twenty-four hours later each well was inoculated with 1.0ml of a virus dilution in medium with 1% FBS. The plates were agitated every twenty minutes for 2 hours then the inoculum was removed. A volume of 3ml 1% Noble agar (Difco) in medium with 1% FBS was added to each well. The plates were incubated at 37°C for 1-3 days according to when the plaques were identifiable, then stained with 10% crystal violet (BDH) in 10% formalin, 5% methanol. After 4 hours the agar overlay was washed off and the plaques were counted.

2.7 Concentration of FCoV

Equal volumes of FCoV stock and cold, neutralised saturated ammonium sulphate (SAS) were mixed and incubated overnight at 4°C. The suspension was then spun at 10,000 rpm for 10 mins. The SN was discarded and the precipitate was resuspended in PBS to about 5% of the original volume. This suspension was then layered onto a SW41 centrifuge tube containing 1.0ml 20% sucrose in PBS on top of 1ml 60% sucrose in PBS. The tube was spun at 35K for 1.5 hrs. The band obtained at the interface of the two sucrose layers was resuspended in 5ml PBS then carefully layered onto a gradient of 2ml 60% sucrose, 2ml 40% sucrose, and 2ml 20% sucrose in PBS which had been standing overnight in an SW41 tube. This was then spun for 2 hrs at 35K. The band of virus which formed at a density of 1.15 $gm.cm^{-3}$ was collected through a hole punctured in the bottom of the tube and was either frozen at -70°C or resuspended in PBS and pelletted by spinning for 2 hrs at 35K, depending on its intended use.

2.8 Protein measurement of FCoV

FCoV protein was quantified by comparison with known concentrations of ovalbumin on PAGE as shown in fig 2.1. Lanes 1-8 show twofold dilutions of ovalbumin, starting at 500 micrograms/ml, the FCoV sample to be measured was neat in lane 10, then diluted twofold (lanes 10-14). The N protein in the last dilution was similar in intensity to the third lane of ovalbumin. Since FCoV at 1:16 was comparable to 125 micrograms/ml of ovalbumin, the concentration of N in this sample was approximately 2.0mg/ml. Similar calculations were made for M and S protein (when visible).

2.9 Indirect immunofluorescence assay (IFA)

FEA cells were inoculated in suspension with an appropriate concentration of the Wellcome strain of FCoV [93] to infect about 50% of the cells. After incubation at 37°C for 4 hours in stirred suspension, the cells were seeded onto 96 well glass plates (C.A. Hendley (Essex) Ltd.) After a further incubation

Fig. 2.1

Measurement of protein content of FCoV by comparison with known concentrations of ovalbumin.

Lanes 1-8	Doubling dilutions of ovalbumin starting at 500 micrograms/ml
Lane 9	Molecular weight markers
Lanes 10-14	FCoV undiluted, and thereafter in twofold dilutions

of 6-8 hours the attached cells were washed in PBS, then fixed, and subsequently stored, in methanol at -20^{o}C. Before use, the plates were washed in PBS.

Test serum was diluted 1:10 and thereafter in doubling dilutions up to 1:1280 in PBS.

The diluted serum was inoculated on to the wells of the 96 well plate and incubated for 60 mins at 37^{o}C. The plates were then washed twice in PBS. Twenty-five ul of fluorescein isothiocyanate conjugated goat anti-cat IgG was incubated on the cells for a period of 60 minutes at 37^{o}C. After further washing in PBS the cells were examined for fluorescence in a microscope using ultra-violet light. The antibody titre was taken as the last dilution of serum which showed fluorescence.

2.10 Polyacrylamide gel electrophoresis

Gels were made using the Mini-protean II dual slab cell (BioRad). The solutions used are listed in table 2.1

For a 5ml gel, the following were mixed from prepared stocks 1.7ml 30% acrylamide: 0.8% bis-acrylamide; 1.25ml 1.5M tris pH 8.6; 2.0ml DW; 50ul 10% SDS and 6.5ul TEMED. Seventeen microlitres of freshly prepared 10% AMPS was added immediately before pouring the gel. An overlay of water saturated butanol was applied and the gel was left to set for approximately 20 mins. The water saturated butanol was poured off, the gel was rinsed with distilled water and dried with blotting paper, and the stacking gel was poured on.

The stacking gel consisted of 600ul of 30% acrylamide:0.8% bis-acrylamide; 750ul 0.75M tris ph 6.8; 1.75ml DW; 7.5ul TEMED; 31ul 10% SDS and 15ul 10% AMPS added last. The comb was added and the gel was left to set at 4^{o}C.

Samples were boiled for 3 minutes in one-third volume RSB or non-reducing sample buffer. A mixture of 2.5ul Amersham

rainbow markers + and 1.3ul RSB was boiled for 1 minute.

Gel running buffer was added between the plates to a level mid-way between the tops of the two glass plates and to the tank. Any trapped air bubbles were removed with a pasteur pipette. The comb was removed and the samples and markers were added.

The mini-gel was run at 200V, 35mA constant current, for approximately 45 mins.

2.11 Immunoblot preparation and development

The proteins were transferred from the gel to nitrocellulose in immunoblotting buffer (see table 2.2) for 1hr at 100V using a Mini Trans Blot (Biorad). The remaining binding sites were blocked with 0.5% non-fat milk powder in TBS for 3 hrs or overnight. The blot was dried and stored at -20^{o}C in parafilm.

The blot was cut into strips and each was incubated with the serum to be tested at a dilution of 1:10 in solution A (see table 2.2) for 2 hrs or overnight. The strip was washed 3 times in PBS/Tween, then 0.004% biotinylated protein A (Sigma) in solution A was added for 1 hr. The strip was washed 3 times in PBS/Tween then 0.001% streptavidin alkaline phosphatase (Southern Biotechnology Associates, Inc.) in solution A was added for 1 hr. After washing 3 times in PBS/Tween, the strip was developed in darkness in 0.0033% BCIP (Sigma) and 0.0066% NBT (Sigma) in AP buffer until bands appeared (usually 10-20 mins).

2.12 FeLV test

Samples were screened for FeLV antigen using an ELISA (FeLV Petcheck, Idexx) to detect p27 antigen and positive results were confirmed by virus isolation [75].

2.13 FIV test

A commercial ELISA, FIV Antibody Test Kit (FIV Petcheck, Idexx) was used to screen samples for FIV antibodies.

2.14 Histopathology

Samples for histopathology were fixed in 10% neutral buffered formalin and embedded in polywax before sectioning. Sections were stained with haematoxylin and eosin. Histopathological examination was kindly performed by Dr Sarah Toth.

CHAPTER 3

THE PREVALENCE OF ANTI-FCoV ANTIBODIES IN SELECTED CAT POPULATIONS

3.1 INTRODUCTION

In the absence of a reliable method of isolating FCoV from cats in the field [45, 102, 110, 164] serology is widely used for diagnostic purposes. However, as discussed in chapter 1, one of the difficulties in interpreting FCoV antibody titres is the presence of antibodies not only in diseased but also in healthy cats [3, 7, 9, 56, 80, 96, 102, 103, 104, 105, 155].

In order to interpret the significance of an antibody titre in a given cat, the background prevalence of antibodies had to be established for comparison. To this end, information was gathered about cats which had died of FCoVV and a survey was conducted to study the prevalence of FCoV antibodies in the U.K.

3.2 MATERIALS AND METHODS

PM material from cats with FCoVV was sent to the FVU by veterinary practitioners in the UK for diagnosis by histopathology. Some cases were referred to the small animal medicine department of the University of Glasgow for diagnosis and, eventually, PM.

FeLV, FIV and FCoV IFA tests were performed as described in Chapter 2. Serum dilutions were made to 1:1280 and therefore this titre represents all titres equal to and over 1280.

Plasma samples from 127 healthy domestic cats were obtained by veterinary surgeons in general practice from cats submitted for neutering or orthopaedic operations [63]. Other sera used were those submitted to the FVU for routine or diagnostic serology or virus isolation. Sera from 100 sick and healthy pedigree cats, 100 sick domestic cats, 88 domestic cats from single cat households (SCH), 100 from domestic cats in multicat households (MCH) were examined by IF for evidence of anti-FCoV antibody. Eighty-six of the healthy pedigree cats were from MCH and the housing of 14 was unknown. Unfortunately there were insufficient pedigree cats from SCH for comparison with other groups.

Statistical analysis was kindly performed by Dr Chris Robertson (Strathclyde University).

3.3 RESULTS

3.3.1 Pathology

FCoVV cases were classified as effusive if over 5 ml of ascitic fluid was present. Five ml was an arbitrary choice, since some leakage into the peritoneal cavity is to be expected at PM if the animal has been dead for more than a few hours. The appearance of a classical case of effusive FCoVV is illustrated in fig.s 3.1 and 3.2. On the omentum of the cat in fig. 3.2 pyogranulomata 1-2mm across are visible. The typical appearance of non-effusive FCoVV granulomata is shown in fig. 3.3.

Atypical pathology was noted with moderate frequency: no macroscopically visible pyogranulomata (fig. 3.4), thickening of the colon not unlike alimentary lymphosarcoma (fig. 3.5), tumour-like lesions on the surface of the intestine and in the mesenteric lymph node (fig. 3.6) and miliary tumour-like appearance in the lungs (fig. 3.7).

3.3.2 Breed

Fig. 3.8 shows the breed distribution in 38 effusive and 23 non-effusive cases of FCoVV. Sixty-three percent of effusive cases were in pedigree cats of which 10 cats (26%) were Persians. Forty-eight percent of non-effusive cases were pedigree cats of which 4 (17%) were Burmese.

The results of the FCoV antibody survey are presented in table 3.1. FCoV antibody was most prevalent in healthy pedigree cats (53%) and least prevalent in healthy domestic cats (14%).

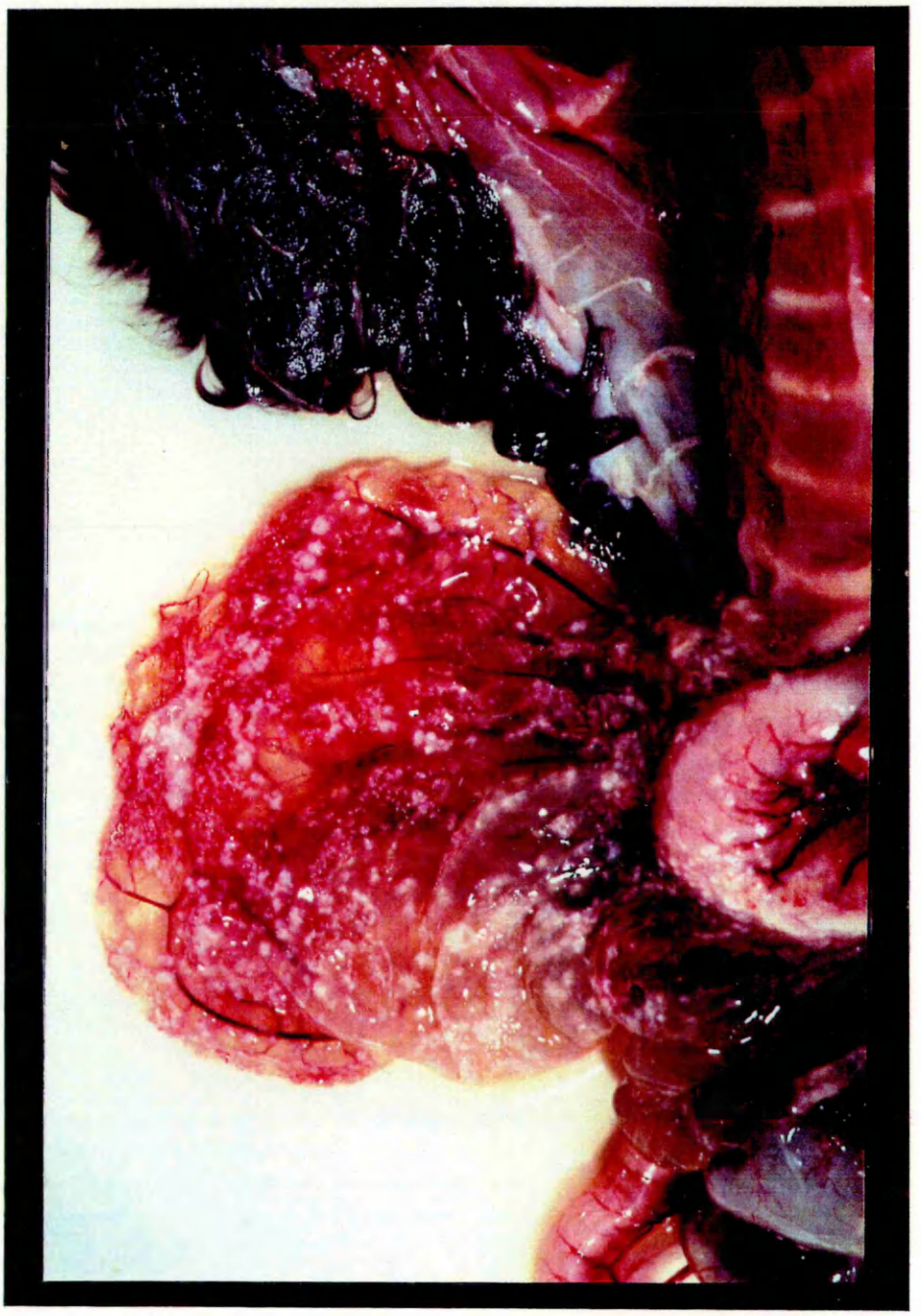
3.3.3 Age

The ages of the animals which died of FCoVV are presented in fig. 3.9. Fifty-five percent of the cats were under a year of age and a further 14% were 12-18 months old. After 18 months of age the incidence was sporadic.

Fig. 3.1

The classical appearance of effusive FCoVV showing the typical straw-coloured effusion and adhesions in the thoracic cavity. Thoracic effusions occur in around 20% of FCoVV cases.

Fig. 3.2



Typical pyogranulomata 1-2mm in diameter are seen on the omentum of a cat with effusive FCoVV.

Fig. 3.3

Pyogranulomata in non-effusive FCoVV on the surface of the kidneys.

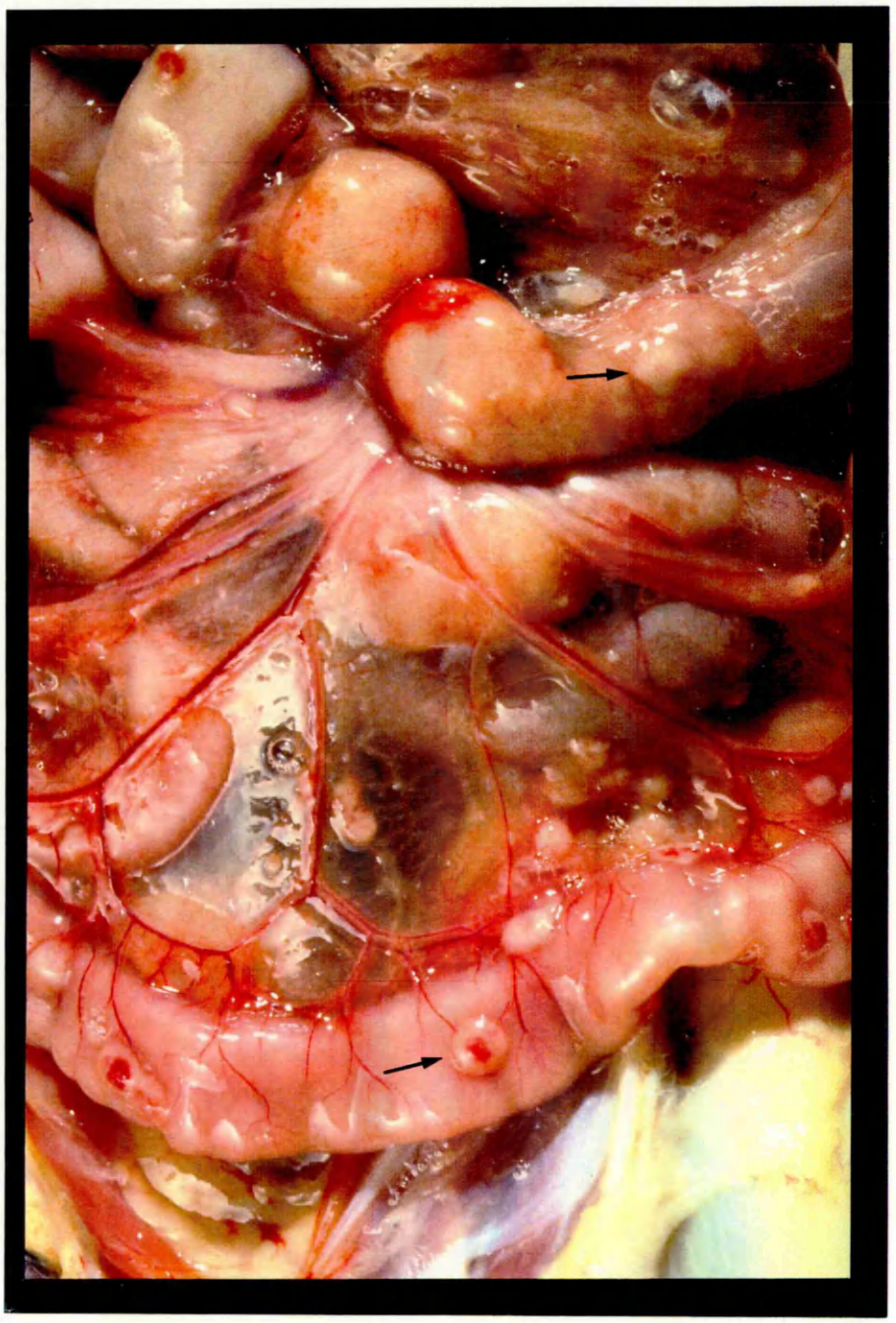
Fig. 3.4

Atypical presentation of FCoVV: no macroscopically visible pyogranulomata or fluid in a confirmed case of FCoVV.

Fig. 3.5

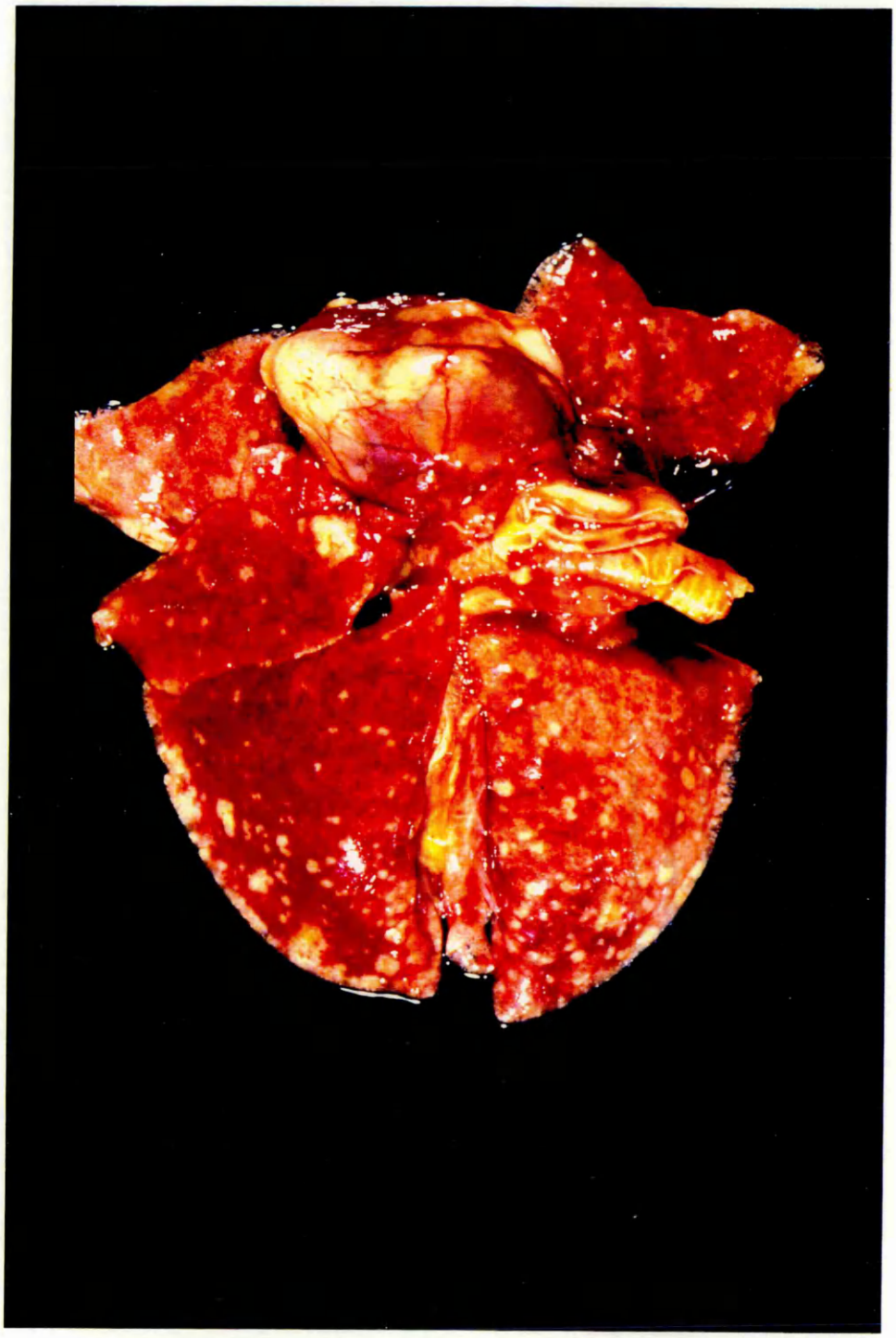
Thickening of the colon due to FCoVV, a lesion which could easily be mistaken for alimentary lymphosarcoma.

Fig. 3.6



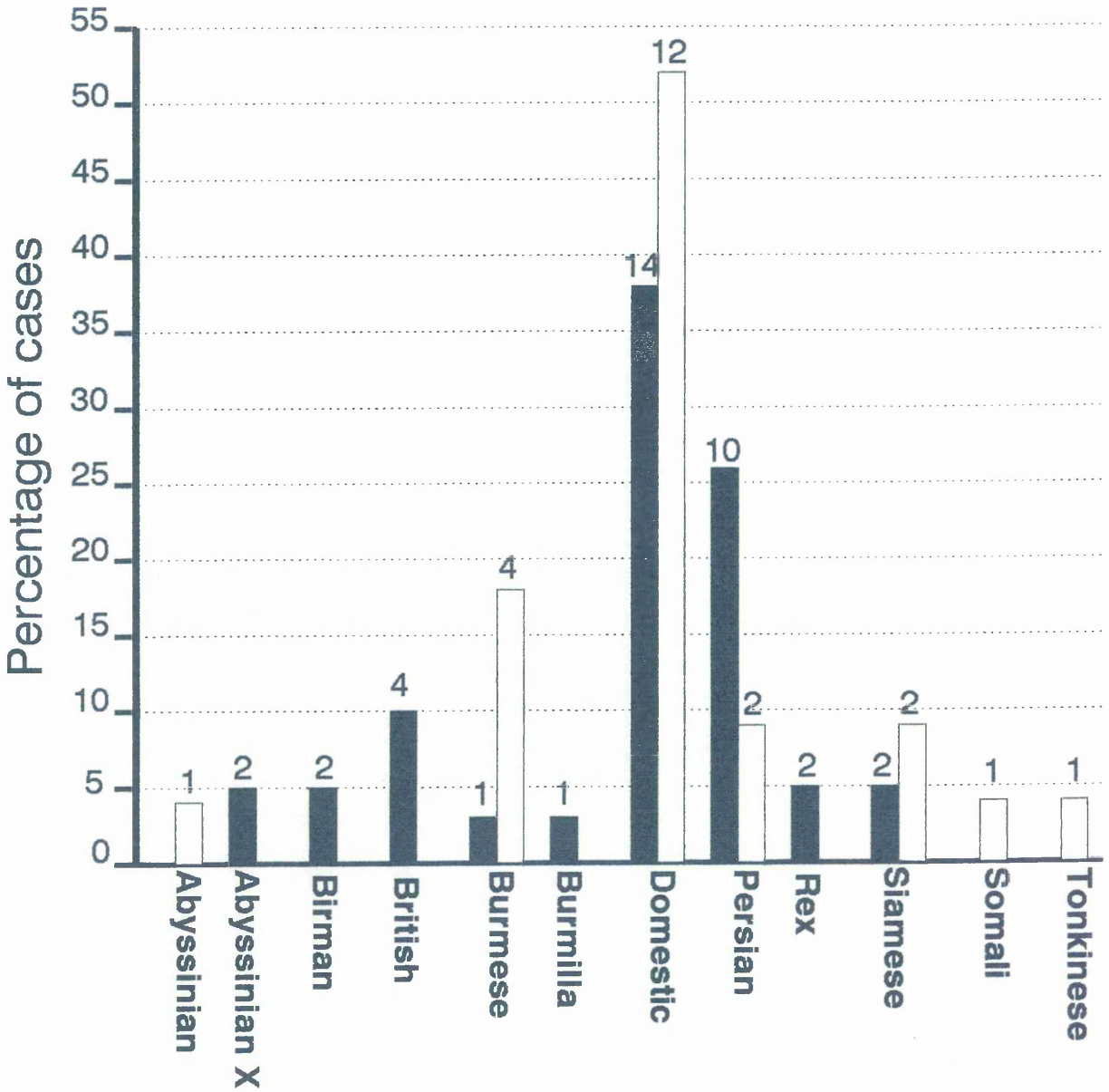
Tumour-like FCoVV lesions on the surface of the intestine and mesenteric lymph nodes.

Fig. 3.7



Miliary appearance of FCoVV pyogranulomata in the lungs.

Fig. 3.8



The breed incidence in 38 effusive and 23 non-effusive cases of FCoV vasculitis

Key:

Effusive

Non-effusive

Table 3.1 Survey of FCoV antibodies in selected groups of cats

	Proportion seropositive
Healthy pedigree	53/100*
Healthy domestic	18/127 (14%)
Sick pedigree	24/100
Sick domestic	20/100
SCH domestics	14/88 (16%)
MCH domestics	28/100

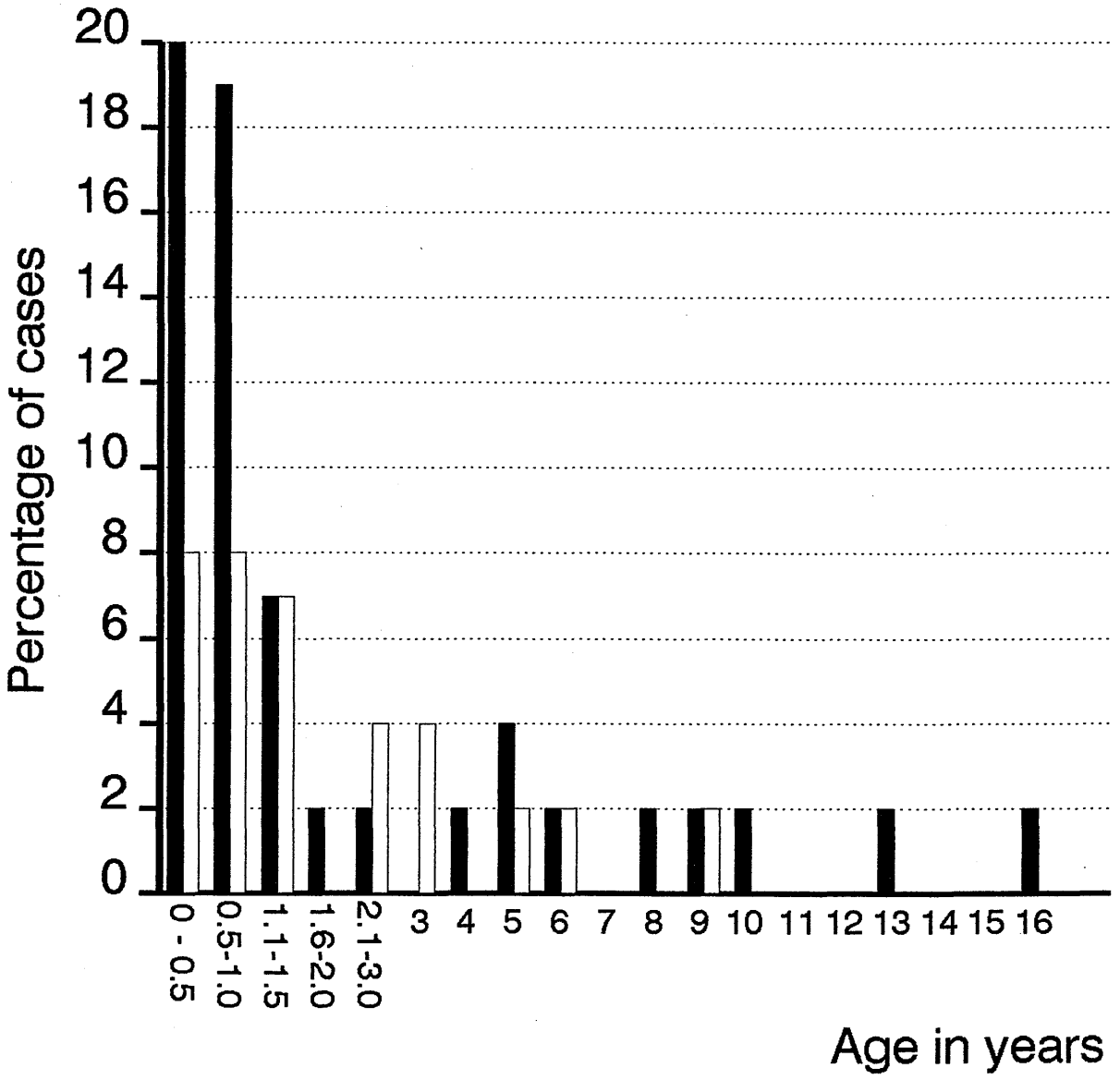
Key:
* number of seropositive cats over total number of cats tested
SCH single cat household
MCH multicat household

Table 3.2 The sex distribution of cases of FCoVV and presence of antibodies to FCoV

	Female	Female neuter	Male	Male neuter
a.				
Effusive	16	2	13	7
Non-effusive	7	5	6	5
b.				
100 sick ped	10/29 (34)	1/10 (10)	8/40 (20)	5/19 (26)
100 sick dom	8/28 (28)	1/10 (10)	5/27 (18)	6/34 (18)
100 healthy ped	28/52 (54)	2/5 (40)	16/32 (50)	3/7 (43)
127 healthy dom	12/68 (18)	0/5 (0)	6/50 (12)	0/4 (0)
88 SCH dom	7/37 (19)	1/11 (9)	4/30 (13)	2/8 (25)
100 MCH dom	13/55 (24)	0/5 (0)	14/35 (40)	0/4 (0)
Total	78/269(29)	5/46 (11)	53/214(25)	16/76(21)

Key:
a. cats with FCoVV
b. no. of seropositive cats from the survey against the total for that sex in each group. Percentage in brackets.
ped pedigree
dom domestic
SCH single cat household
MCH multicat household

Fig. 3.9



Age distribution in 59 cases of FCoV vasculitis

Key:

Effusive

Non-effusive

Cats' ages were available for 85 domestic cats from SCH: 119 healthy domestic cats and 93 domestic cats in MCH. No ages were available for the sick domestic or healthy or sick pedigree cats. The results are presented in fig. 3.10. Forty-three of 228 (19%) cats under 3 years old were seropositive compared with 7/69 (10%) of cats over 3 years old.

3.3.4 Sex

The sex of cases of FCoVV is presented in table 3.2 a. Overall, there were 30 females and 31 males. Four more cases, where the type of FCoVV was unknown, were male. There was no difference in sex distribution between effusive and non-effusive forms.

In table 3.2 b. the sex distribution of seropositive and seronegative cats is recorded. In total, 26% of female and 24% of male cats tested were seropositive.

3.3.5 FeLV/FIV

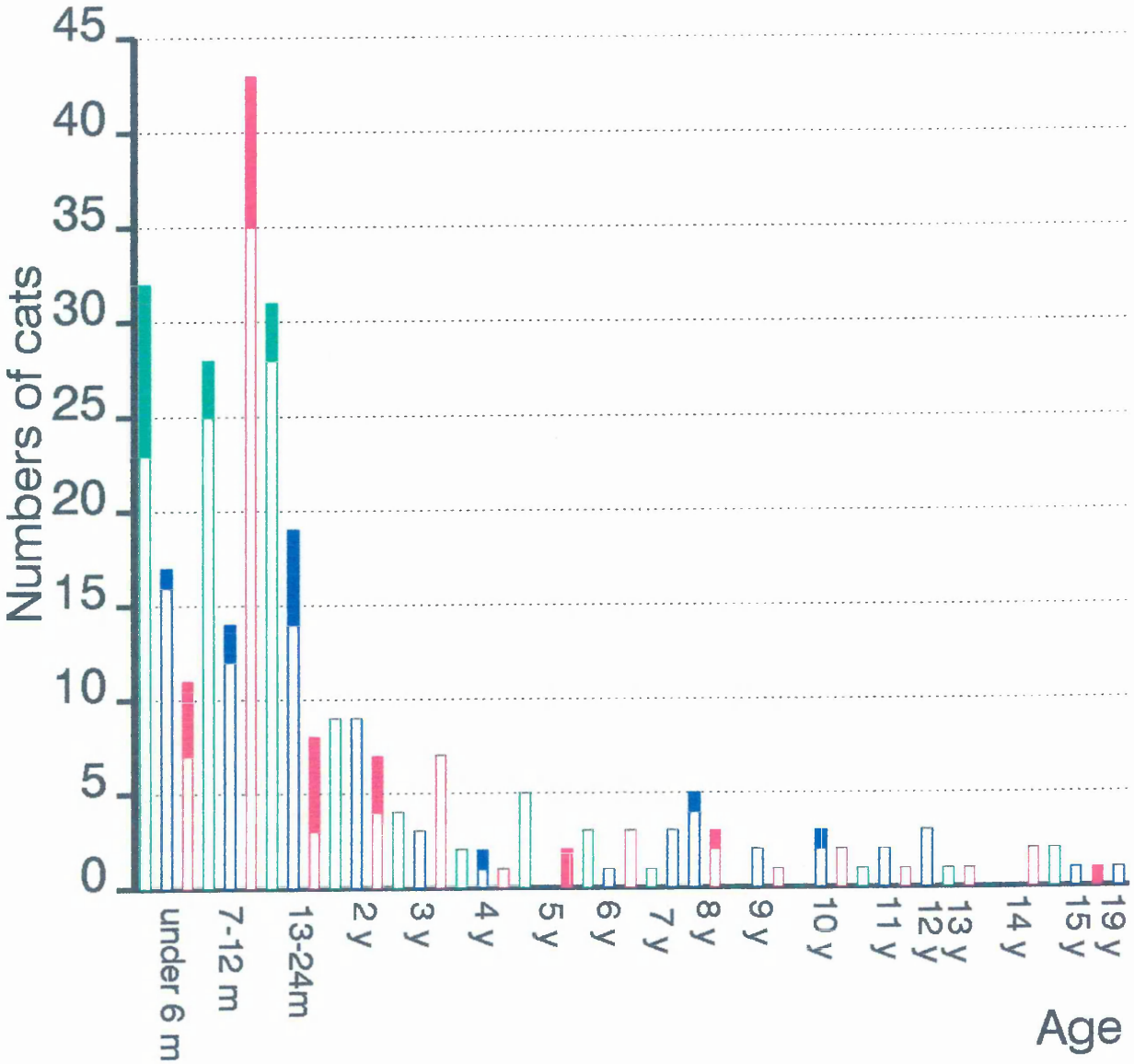
The prevalence of FeLV and FIV in cases of FCoVV is presented in table 3.3.

In table 3.4, the prevalence of FeLV and FIV infection in the 6 different categories is given along with the number of seropositive cats in brackets. Numbers were too low to be very meaningful but the proportion of FCoV seropositive cats with FeLV/FIV was the same as in the general population.

3.3.6 Antibody levels

The antibody titres of cases of effusive and non-effusive FCoVV cases are presented in fig. 3.11 and in different categories of cats are presented in fig. 3.12. It can readily be seen that titres are generally lower in healthy domestic compared to healthy pedigree cats and in SCH as opposed to MCH.

Fig 3.10



Age range in FCoV antibody survey showing antibody status of cats.

Key:

Seronegative

Seropositive

Healthy domestic cats

Domestic cats from SCH

Domestic cats from MCH

Table 3.3 The incidence of FeLV and FIV in histopathologically confirmed cases of FCoVV

	Effusive			Non-effusive		
	Total no. cats	No. pos.	%	Total no. cats	No. pos.	%
FeLV	26	3	11.5	22	4	18
FIV	24	2	8	19	2	10.5

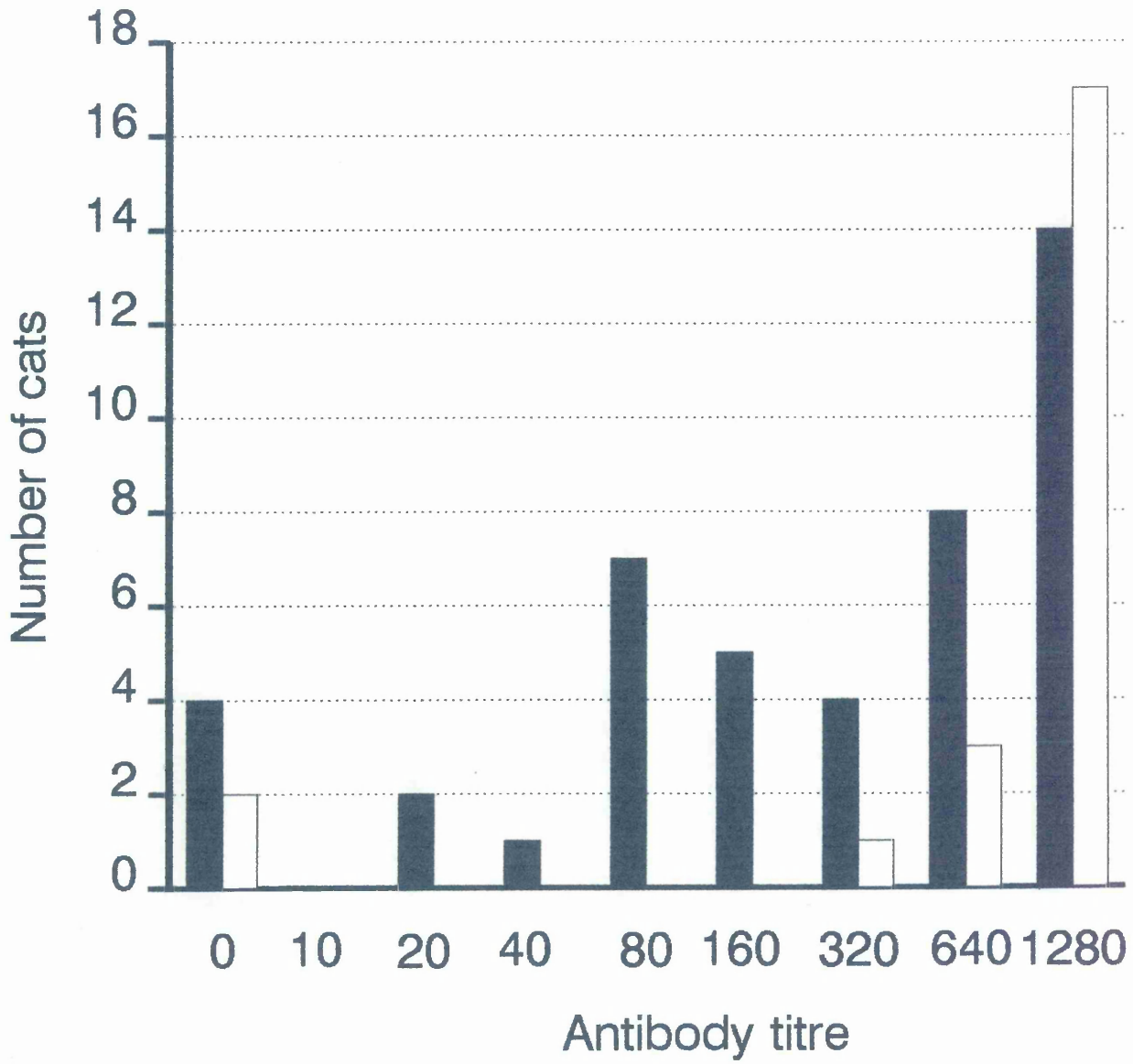
Table 3.4 The prevalence of FeLV and FIV in the selected populations in the serosurvey

	FeLV+	FIV+
Healthy pedigree*	2 (1)	8 (5)
Healthy domestic	4 (2)	3 (0)
Sick pedigree*	8 (2)	9 (3)
Sick domestic**	15 (6)	26 (5)
SCH domestics	4 (0)	5 (2)
MCH domestics	2 (1)	5 (0)

Key:

FeLV+	Feline leukaemia virus positive
FIV+	Feline immunodeficiency virus positive
()	number seropositive
SCH	single cat household
MCH	multicat household
*	1 had both FeLV and FIV
**	5 had both FeLV and FIV

Fig 3.11

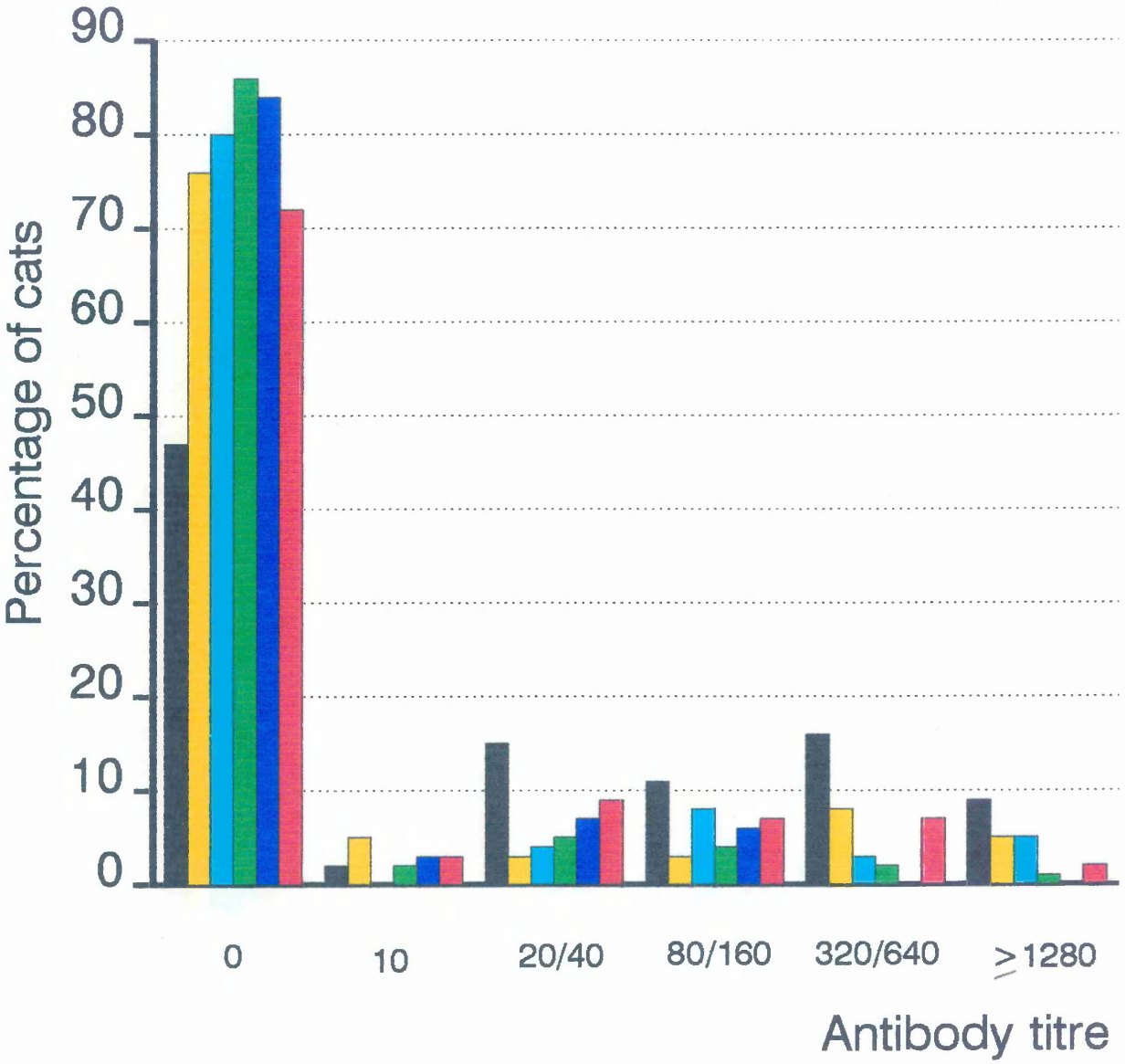


Antibody titre of cases of FCoV vasculitis

Effusive

Non-effusive

Fig 3.12



The range of antibody titres in selected groups of cats.
Given as a percentage when necessary.

Key:

- 100 healthy pedigree
- 100 sick pedigree
- 100 sick domestic
- 127 healthy domestic
- 88 domestic from SCH
- 100 domestic from MCH

3.3.7 Housing

Information on the housing of the cats which died of FCoVV was only available for 40 animals, 36 were from MCH and 4 from 2-cat households.

In the FCoV antibody prevalence survey, 114 healthy domestic cats were free-ranging, 6 were kept indoors and the status of the others was unknown. Eight SCH domestic cats were kept indoors and 77 were free-ranging. Of the MCH domestic cats, 3 were kept indoors and 95 were free-ranging. This information was unobtainable for the other three categories. There were insufficient indoor cats in these categories to establish if being free-range or indoor made a difference to the cat's chance of being seropositive.

Domestic cats were more likely to be seropositive if from MCH than SCH but it was not significant ($P = 0.6$). Pedigree cats were 3 times as likely to have antibodies in a MCH than in a SCH.

3.4 DISCUSSION

3.4.1 Pathology

There were sufficient numbers of FCoVV cases with atypical gross pathology to justify the assertion that all cases should be confirmed by histopathology. Resemblance to lymphosarcoma associated with FeLV has been noticed previously [87, 101]. The colonic form of FCoVV has been reported before and can exist on its own or in association with more typical signs of FCoVV [147].

3.4.2 Breed and housing

Previous surveys of cats which had died of FCoVV or of cats which had antibodies against FCoV have given conflicting opinions about breed predilection.

It was interesting to note that the non-effusive form was found predominantly in domestic cats, while the effusive form was encountered more frequently in pedigree cats. The predominance in pedigree cats could be due either to genetic predisposition or the fact that they are more often kept in large groups [2, 105]. The higher incidence in Persians, together with the predominance of the effusive form in that breed may indicate a genetic susceptibility. Higher incidence in Persians has been noticed before [12] as has higher incidence in Burmese [140] which was the second commonest pedigree breed in this survey.

From this survey, it was clear that both cases of FCoVV and FCoV antibodies were more prevalent in MCH. Ninety percent of FCoVV cases, where housing status was known, had come from MCH. Increased prevalence in MCH has been noted before [2, 12, 80, 102].

Healthy pedigree cats were twice as likely as any other group to have FCoV antibodies. Eighty-six were known to be from MCH and 46 of these (53%) were seropositive. Only 28% of domestic cats from MCH were seropositive. It is unlikely that being

pedigree or not alters the predisposition of a cat to seroconvert in response to an antigen, though inbreeding may alter the immune response. Possibly pedigree cats are more prone to becoming FCoV carriers.

In this survey it was established that young cats are more likely to be seropositive than older cats. Perhaps pedigree cats in MCH are in general younger since they tend to be kept for breeding. However, it is likely that life-style of the pedigree cat, going to shows and exchange of cats between households for mating, increases both the frequency of exposure to FCoV and the virus dose. This might explain why sick pedigree cats had a lower prevalence of antibodies than did healthy pedigrees: possibly these activities are suspended for a while if the animal is ailing. Alternatively, the nature of the cats' illness may depress the immune response or the antibody may be consumed in the pathological process. The housing of these sick cats was unknown. Perhaps many were from SCH and therefore not exposed so often to FCoV.

The levels of antibody were higher in pedigree compared to domestic cats which may represent current or recent exposure to virus or an anamnestic response to repeated exposure. It has been suggested that cats with higher titres are more likely to be FCoV excretors [111]. This question will be addressed in Chapter 5.

3.4.3 Age

In this survey, 58% of effusive and 43% of non-effusive cases of FCoVV were under one year of age. The slightly later peak of non-effusive FCoVV cases could be a reflection of the more developed immune system of older cats because the non-effusive form is thought to be an intermediate stage between the effusive form and immunity. Alternatively, it may only reflect the longer incubation period of this form. In this survey, there was an abrupt decline of FCoVV incidence after 1.5 years

of age, which was earlier than in previous surveys [102, 140].

It is tempting to assume that FCoVV occurs more frequently in younger cats because their immunity is less mature, but how much multicat housing and the stress of rehoming influences this is unknown. Clearly the fate of older seropositive cats in MCH needs to be examined. Increased susceptibility to FCoVV in experimental cats with pre-existing antibody has been reported [49, 109, 112, 156, 157, 158]. If this were also true in the field, one would expect a higher incidence in older cats which had been exposed to FCoV more than once. Surprisingly, in this survey, cats under 3 years of age were twice as likely to be seropositive as those over 3 years old.

3.4.4 Sex

No sex predilection was noted in either those cats which had died of FCoVV or had seroconverted.

3.4.5 FeLV/FIV

In this survey of cases of FCoVV, the prevalence of FeLV was 14.6% which was not significantly different from the prevalence of FeLV in sick cats in the U.K. [63]. This result agrees with more recent surveys which showed that the incidence of FeLV in cases of FCoVV is much the same as in the rest of the feline population [118, 140].

The prevalence of FIV was 9% which was significantly less than the prevalence of 19% in sick cats in the UK [63]. This finding agrees with that of a previous survey [69]. One explanation could be the differing epidemiological patterns between the two infections: FCoVV was found to affect young pedigree cats whereas FIV increases in prevalence as age increases and is less likely to be found in pedigree cats [63]. None of the FIV positive cats in this survey was pedigree.

3.4.6 Antibody levels in FCoVV cases

Most cats which die of FCoVV have antibody titres equal to or over 1280. Some effusive cases had lower titres. FCoVV cases with no detectable FCoV antibodies have been reported and were also found in this study. Several explanations were reviewed in 1.9.5.

Recovery of virus from cases of FCoVV is extremely difficult [45]. Even in experimental infections with a laboratory strain of FCoV, virus could only be detected for 2-16 days p.i. [140,141]. For antibody production to continue, antigen must continue to be present.

There is good evidence that FCoVV is an immune complex mediated disease [57, 60, 70, 71, 102, 109, 154, 156, 157]. Immune complex formation depends on a particular balance of antigen and antibody, neither being in excess, therefore it is not the magnitude of the antibody response that is important but the antibody:antigen ratio. This might be the reason why in the more rapidly developing, effusive, manifestation of this disease antibody levels were sometimes quite low.

The vasculitis resulting from immune complex deposition in blood vessels causes the clinical signs we associate with FCoVV. Even if immune complexes are present for only a short time before the onset of clinical signs they could cause such severe damage to the animal's vascular system that it cannot recover. This subject will be addressed again in Chapters 4 and 6.

CHAPTER 4

THE RELEVANCE OF ANTI-FCoV ANTIBODIES TO THE FATE OF CATS NATURALLY EXPOSED TO THE VIRUS.

4.1 INTRODUCTION

The fate of cats with antibodies to FCoV is of major concern to cat owners, particularly breeders, and their veterinary surgeons. Of particular importance is the significance of the presence of antibodies in healthy cats exposed to a case of FCoVV.

The IFA test has been used as an aid to diagnosing FCoVV and as a screen to determine if a household is infected with, or has been exposed to FCoV [3, 9, 155]. However, once a household has been found to have FCoV antibodies, many more questions arise:

1. Does the presence of FCoV antibodies indicate FIPV or FECV infection?

2. How many more cats can be expected to die from FCoVV?

3. Is any particular antibody titre or pattern of titres indicative of this fate?

4. What period elapses between seroconversion and death and at what stage, if ever, can one assume that a seropositive cat is immune?

5. Will a seropositive cat suffer enhanced disease if reinfected?

6. Will the cat's antibodies ever disappear? If so, in what time scale?

7. What factors, if any, determine whether cats become seronegative, stay seropositive or develop FCoVV?

8. Can cats which have become seronegative begin to excrete virus again?

9. Is a cat which is FIV or FeLV positive more likely to succumb to FCoVV?

In this chapter, a survey is described in which seropositive cats and their seronegative in-contacts were followed for periods of up to 3 years. Households were chosen with different clinical histories so that hopefully strains of FCoV of varying pathogenicity could be examined. It was found that there were cases of FCoVV in cats from households with all types of clinical history indicating that the assumption that a household has a FECV or non-pathogenic FCoV infection on grounds of clinical history is unwise.

Death rates from FCoVV appeared to be higher (around 12%) in index infections than in households which had been seropositive for some time (around 3-4%). Repeat IFA titres were of little value in predicting a cat's fate since even some cats which appeared to have recovered from infection and became seronegative later died of vascular lesions sustained during the time of active infection. These lesions were mainly in the kidneys. By contrast, there were no cases of FCoVV in cats which did not seroconvert throughout the survey. It will still be some time before the true mortality rate of FCoV infection can be assessed. It may be that FCoV infection is responsible for some of the presently unexplained kidney disease of the older cat.

The infection was eliminated from some households and all of the cats became seronegative. On reinfection, these cats and cats whose antibody titres had fallen considerably and rose again appeared not to show ADE because the mortality rate was still

very low. Households where cats were kept in groups of 3 or less were more likely to eliminate the infection than those which maintained cats in larger groups.

In households that became seronegative, when reinfection occurred the source of virus could always be traced to contact with a cat which was seropositive or of unknown FCoV antibody status. There was no evidence to suggest that seronegative cats could be carriers of FCoV.

Seven households had concurrent FeLV infection and 6 had concurrent FIV infection (of which, one household had both). The incidence of FCoVV was no higher in FeLV infected households than in non-infected households. The presence of FIV in a group of cats slightly increased the chance of a death due to FCoVV.

4.2 MATERIALS AND METHODS

4.2.1 Seropositive household survey

In 1988, a survey was begun to monitor the antibody status of cats in FCoV seropositive households. Seventy-two cat owners participated, with over 700 cats. Household size ranged from 1-42 cats.

In table 4.1, the reasons for household participation are presented. Diagnosis of FCoVV was confirmed by histopathological examination where possible. Presumptive diagnosis of FECV infection was made according to clinical history as described by Dr Niels Pedersen: the presence of non-responsive diarrhoea primarily in kittens 5-12 weeks of age [103, 110, 111, 114]. FECV was given as a histopathological diagnosis by Dr Irene McCandlish in one household (1598) due to stunting and fusion of the SI villi similar to TGEV infection in piglets.
Most cats were bled and necropsied by their veterinary surgeons. The cats in households 1598, 1786, 1801 and 2863 were bled and necropsied by the author.

The health of cats in all of the households except 1688 were checked by telephone in September 1990.

A computer database was constructed to record the sequential blood samples from each cat. The data comprised concurrent FeLV and FIV results, age, health status, whether free-ranging or kept indoors, and from MCH or SCH. If from the former, the number of in-contact cats and the highest antibody titre of in-contact cats where known, were recorded.

4.2.2 IFA

Antibody measurement was performed by IFA as described in 2.9. Since consecutive tests on any cat were performed at varying intervals over the three year period, it was important to assess whether fluctuations in antibody titre arose because of

Table 4.1 Reasons for participation of households in the survey and FeLV and FIV test results.

Owner ref	Reason 1st sampled	No. FCoVV	Dead other	FeLV	FIV
Cats died of FCoVV on premises					
216	1 SA	0	0	-	+
1594	1 S	1 SK	0	-	N/D
1596	1 CA	0	0	N/D	N/D
1613	4 SA	0	2	-	N/D
1615	1 S	2 CA	0	+	-
1627	2 SA	0	0	N/D	N/D
1673	2 S	0	2	+	N/D
1674	1 S	0	0	-	N/D
1677	1 SK 1SA	3 CA	9	+	+
1678	1 S	0	1	-	N/D
1680	1 CA	0	1	-	-
1682	1 S	0	1	N/D	-
1683	1 CA	0	3	N/D	N/D
1690	1 CA	0	0	-	N/D
1692	1 S	0	0	-	N/D
1693	1 SA	0	1	-	-
1694	1 SA	1 SA	0	+	N/D
1768	2 SA 1SK	1 CA 1CK 1SK	1	N/D	N/D
1769	1 SA	0	1	-	-
1770	1 SA	1 S	1	N/D	N/D
1773	1 S	0	1	N/D	N/D
1777	1 S	1 CA 1SK	3	-	+
1778	1 SA 1SK	0	0	-	-
1781	1 CA	1 CA	1	-	-
1784	2 SA	0	3	+	N/D
1785	1 SA	0	0	N/D	N/D
1790	1 SA	1 SA	0	-	-
2097	1 SA	0	0	-	N/D
3122	1 CA	0	1	N/D	N/D
3123	1 CA	0	2	N/D	N/D
3124	1 C 2S	0	0	-	-
Sold kittens died of FCoVV					
1672	SK	2 SA	1	N/D	N/D
1675	2 SK	0	0	N/D	N/D
1684	1 SK	1 CA	1	-	N/D
1686	2 SK	0	0	N/D	N/D
1691	SK	0	1	N/D	N/D
1771	1 CK	1 CA	1	-	+
1775	1 SK	0	1	-	-
1776	1 CK 2SK	1 CA	1	-	-
1779	1 SK	0	0	-	-
1789	SK	2 CA 1SA	3	-	-
1801	2 CK	0	0	N/D	N/D
3117	2 SK	0	3	-	-
3121	1 CK	0	0	-	-
3125	1 CK	0	0	N/D	N/D

contd.

Table 4.1 contd.

Owner ref	Reason 1st sampled	No. FCoVV	Dead other	FeLV	FIV
Clinical signs suspicious of FCoV infection					
54	S clin.case	0	0	-	N/D
1685	R (flu)	1C 1S	11	-	+
1772	R (E)	0	0	N/D	N/D
3095	S (preg)	1 CK	0	-	-
Clinical history indicative of FECV infection					
1598	E C	3 CA 2CK	0	-	-
1612	E	0	0	N/D	N/D
1676	E	1 CK	1	-	-
1780	E	0	4	N/D	N/D
In contact with real or suspected FCoV excretor					
1614	IC S	0	1	N/D	N/D
1681	IC S	0	1	N/D	N/D
1687	IC KS	0	0	N/D	N/D
1689	IC S	1	1	-	N/D
1774	IC S	0	1	-	-
1788	IC S	0	3	N/D	N/D
2439	IC	0	0	N/D	N/D
2863	IC C	0	0	N/D	N/D
No history indicative of FCoV infection					
1595	R	0	2	-	-
1679	R	0	2	N/D	N/D
1695	R	1 CA	0	-	N/D
1782	R	0	0	-	-
1786	R	0	0	-	-
3119	R	0	0	-	N/D
3120	R	0	1	+	N/D
Reason for first sampling unknown					
3		0	1	N/D	+
1688		0	0	N/D	N/D
1783		0	1	+	-
1787		1 S	0	N/D	N/D

S = suspected of FCoVV
C = histopathologically confirmed
A = adult
K = kitten
IC = in contact with a case
E = enteritis, FECV suspected
R = routine, e.g. for mating, no cases FCoVV.
N/D = not done
\+ = positive
\- = negative

genuine differences in the cat's antibody level or reflected experimental variation. To this end 45 samples from 10 cats were retested and the plates were read by the 3 persons who usually performed the tests (Mrs J. Simpson, Mr M. Golder and the author). The results are presented in table 4.2. The new results were compared with the original results. Statistical analysis, kindly performed by Dr Chris Robertson of Strathclyde University, showed that there was no evidence of any significant difference among the three readers. Variation between titres was random. Once differences among the samples and cats had been taken into account, the standard deviation of the log (base 10) values was 0.48. This result showed that variation in IFA titres reflected real fluctuations in antibody levels in the cat.

At the beginning of the survey, testing was monthly. However, this frequency met with cat owner resistance and since little change was noticed in IFA titres from month to month, testing thereafter was at intervals of 3-6 months or whenever the owner would permit.

4.2.3 FeLV/FIV

The adult cats in 43 households were screened for FeLV and in 29 households were tested for FIV using the tests described in 2.12 and 2.13.

Table 4.2 Comparison of three readings of sequential IFA titres from 10 cats

The original IFA titre is given first and the rechecks are below. The original might have been read by Mrs Simpson, Mr Golder or the author. The first recheck was by the author and the second by Mr Golder. The two recheck readings are on the same plate.

5678*

P2405C**	P5527C	P7621C	P9385C
40	80	320	80
160	320	320	160
160	320	320	160

5542

P954C	P3548C	P8340C	P3580D
80	320	40	320
640	320	40	160
320	320	20	160

5691

P1131C	P1617C	P2495C	P5702C	P7125C	P8926C	P1995D
640	1280	640	320	160	1280	640
320	1280	640	640	20	160	320
320	1280	640	320	40	160	320

5692

P1134C	P2492C	P4051C	P5701C	P7128C	P8931C	P1999D
160	320	320	80	20	1280	160
40	320	320	160	20	640	160
40	320	320	80	20	160	80

5732

P4002C	P5976C	P9113C
40	320	80
160	160	640
40	160	640

5733

P4011C	P5974C	P9111C
0	40	0
80	20	80
20	20	40

* Cat's reference number
** Sample reference number

Table 4.2 contd.

5734

P4007C	P5979C	P9105C
20	160	0
20	160	0
20	80	40

5011

P7997B	P1650C	P5902C	P9437C
0	80	0	320
0	0	0	20
0	0	0	0

5187

P225C	P2712C	P7334C	P35D
40	40	320	40
40	20	0	40
40	40	40	80

5361

P7744B	P8336B	P168C	P2996C	P5898C	P8489C
320	160	160	320	80	640
160	80	320	80	80	20
320	160	320	320	160	320

* Cat's reference number
** Sample reference number

4.3 RESULTS

4.3.1 The prevalence of FCoVV in households with different clinical histories

The reasons for first sampling cats for FCoV antibodies are presented in table 4.1. Households broadly fell into 5 categories: those which had a case of FCoVV on their premises, or in a kitten which was bred in the household and had been sold to another owner; those which suspected FECV infection; those which suspected contact with an excretor of FCoV; and those with no history of contact or illness.

It can readily be appreciated from table 4.1 that deaths due to FCoVV occurred in cats from all categories. There were 7 further deaths in the 31 households which had had a death within their household; 4 further deaths in the 14 households which had had a sold kitten die; 5 confirmed cases of FCoVV in household 1598 which had the histological report of FECV and one in another household with a clinical history suggestive of FECV. There were 2 deaths in cats from households which had been tested because cats had symptoms which might have indicated FCoV infection; one death from the 8 households which had no history of FCoVV or FECV; and one from the 4 households where the reason for first sampling was unknown.

Thus it was concluded that under no circumstances can the presence of FCoV in a household be judged to be non-pathogenic on the basis of clinical history or even histopathological evidence that the infection produced enteric lesions.

4.3.2 The mortality rate in FCoV infected cats

In an attempt to calculate the mortality rate of a FCoV infection newly introduced into a household, the proportion of cats which died of FCoVV was obtained by dividing the number of cats which died of FCoVV initially (39) by the total number of cats in those households which presented for testing because of a FCoVV death within the household (331): this gives a

proportion of 12%. If seropositive cats alone are counted (39/224) a mortality rate of 16% is found. In Chapter 6 it will be shown that cats with an IFA titre of 0 may have anti-N antibody which can be demonstrated by immunoblotting, therefore the true number of infected cats is impossible to determine by IF alone. The reason the other groups were excluded from this calculation is because they may have had FCoV in their household for some time. Of course, some of the group included may have had FCoV infection for some time but in the absence of a serological history there was no way of discovering this.

There were 11 subsequent deaths in this group: 2 in households which subsequently became mainly seronegative, and one in a house which became mostly seronegative then seropositive again. Hence, 11 divided by the total number of cats at the start of the survey plus the new cats (and excluding those which had died of FCoVV at the start of the survey) (325) gives a figure of 3% for further mortality.

To obtain the mortality rate amongst seropositive cats, the total number of subsequent FCoVV deaths in adults from all groups was divided by the total number of seropositive cats at the beginning of the survey (not counting the adult cats which died of FCoVV at the start). The proportion obtained was 26/599 (4%).

Eighty cats (13%) died from other causes. Since histology was available for only 14 (2%) of which 2 were inconclusive, it was only in 2% that there was no sign of FCoVV. These results are presented in table 4.3.

Ninety-seven cats were lost to the survey because they were re-homed.

Table 4.3 The causes of death in 80 survey cats which died for reasons other than FCoVV

No. cats	Cause of death	Number which were examined histologically
10	kidney failure	2 (focal interstitial nephritis nephritis and cholangitis)
7	road accident	1 (histopathology inconclusive)
6	FeLV	2 (myeloid leukaemia)
5	FIV	2 (thymoma squamous cell carcinoma)
1	FeLV and FIV	
4	mammary tumours	
3	killed by dogs	1 (proliferative cholangitis and mesenteric lymphadenitis)
2	FUS	
2	cardiomyopathy	2 (cardiomyopathy)
2	euthanased because of loss of house training	
2	euthanased because seropositive	
1	lungworm	1 (lungworm)
1	cirrhosis	1 (cirrhosis)
1	pneumonia	1 (pneumonia)
1	feline calicivirus	
1	shot	
1	toxaemic mastitis	
1	anaesthetic death	
1	gingivitis	
1	ruptured gall bladder	
1	heart failure	
1	eczema	
1	euthanased because aborted	
1	masses in kidney and alimentary tract	
23	unknown	1 (histopathology inconclusive)

4.3.3 Sequential IFA titres in cats which developed FCoVV

Thirty-five cats and kittens died of FCoVV during the course of the survey (excluding index cases) and the IFA records of 21 of these are presented in table 4.4. In the other 14 cases either only one or no samples were available. From this table it can be seen that there is no consistent pattern of IFA response which could be considered characteristic of development of FCoVV. Cats 5040, 5153, 5164 and 5508 which became seronegative were thought to have recovered from the infection, but histopathology revealed lesions consistent with FCoVV in the kidneys of all four and also in the eyes and meninges of cat 5508. These lesions resulted in the death of these animals due to kidney failure in cats 5040 and 5164, and neurological deficiencies in cat 5508. Cat 5153 had a squamous cell carcinoma in the mouth and anaemia as well as FCoVV lesions. Cat 5164 was FIV positive and the others were FeLV and FIV negative. On the basis of clinical and IFA titre history it is therefore impossible to distinguish recovered cats from those which will succumb.

The case records of household 1598, which lost most cats to FCoVV, are presented as an example in table 4.5 to show that the records of the cats which are still well in October 1991 are similar to the records of those which died.

4.3.4 The time course from infection to death

It can also be seen from table 4.4 that the time course from seroconversion to onset of clinical signs is very variable. Eleven cats had been seropositive for over a year before they developed clinical signs resulting from the FCoV infection. Cats 5149, 5038, 5047 and 5684 were seropositive for around three years before they died. Cat 5040 had been seronegative for 15 months before he died of the kidney lesions sustained during active FCoV infection. Another 10 cats died of kidney failure. Histopathology was only available for two and they had no FCoVV lesions. Other survey cats have been reported to have had clinical signs of kidney disease which have resolved

Table 4.4 IFA titre records of cats which died of FCoVV

Cat ref.	House hold	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre
5038	1598	Apr 88 320	Oct 88 160	Jun 89 320	Oct 89 320	Feb 90 1280	Jun 90 >1280	
		Sep 90 1280	Nov 90 1280	died Nov 90				
5040	1598	Apr 88 40	Oct 88 20	Jun 89 10	Oct 89 0	Feb 90 10	Jun 90 0	Sep 90 0
		died Sep 90						
5047	1598	Apr 88 320	Oct 88 160	Jun 89 160	Oct 89 320	Jan 90 80	Jun 90 20	Nov 90 1280
		Apr 91 1280	Jun 91 1280	died Jun 91				
5769	1598	Jun 89 640	Jul 89 1280	Aug 89 1280	Oct 89 640	Jan 90 320	Jan 90 1280	Feb 90 640
		died Mar 90						
5080	1615	Jan 88 160	Mar 88 640	May 88 1280	died Jan 89			
5097	1615	Mar 88 1280	Apr 88 >1280	Feb 89 1280	Mar 89 1280	died Mar 89		
5149	1677	Jul 88 40	Oct 88 20	Apr 89 80	Sep 89 80	Mar 90 320	Sep 90 320	Jun 91 1280
		died Jun 91						
5153	1677	Jul 88 40	Oct 88 0	Apr 89 0	died Jun 89			
5164	1677	Jul 88 10	Oct 88 80	Apr 89 20	Sep 89 0	died Nov 89		
5376	1685	Feb 88 320	May 88 1280	Aug 88 1280	died Aug 88			
5377	1685	Feb 88 1280	May 88 1280	died May 88				

Table 4.4 contd.

Cat ref.	House hold	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre	Date Titre
		Oct 86	Jan 87	Feb 87				
5225	1684	1280	1280	1280	died Feb 87			
		Apr 88	Jul 88	Oct 88	Apr 89	Dec 89		
5508	1689	320	80	160	80	0	died Apr 90	
		Mar 88	Jun 88	Jul 88	May 89	Aug 89		
5340	1768	40	320	40	320	160	died Aug 89	
		Mar 88	May 88					
5412	1770	640	1280	died Jun 88				
		Sep 88	Jan 89	Apr 89	Sep 89	Mar 90	Oct 90	Apr 91
5684	1771	320	320	1280	640	1280	1280	640
		Oct 91						
		>1280	died Oct 91					
		Jul 88	Oct 88					
5466	1776	20	1280	died Oct 88				
		Apr 88	Jun 88	Aug 88	Oct 88	Dec 88	Feb 89	
5481	1777	80	320	320	1280	1280	1280	
		died Mar 89						
		Nov 90	Jan 91					
Brandy	1781	1280	320	died Jan 91				
		Jan 88	Mar 88	Jun 88	Oct 88	Feb 89		
5611	1789	640	320	320	80	1280	died Feb 89	
		Nov 87	Feb 89					
5660	1789	80	1280	died Feb 89				

Table 4.5 Sequential IFA tests on cats in household 1598 showing that IFA sequences are indistinguishable between cats which survive FCoV infection and those which succumb.

Cat ref. no.	Apr 88	Oct 88	Jun 89	Oct 89	Feb 90	Jun 90	Sep 90	Nov 90	Apr 91	Jun 91			
5038	320	160	320	320	1280	>1280	1280	1280	died Nov 90 effusive FCoVV				
5039	160	40	20	80	80	640	1280	160	0				
5040	40	20	10	0	10	0	0	died Sep 90 FCoVV lesions in kidney					
5041	160	160	80	80	160	80	320	160	40				
5042	160	40	20	0	160	20		640	40				
5043	40	80	80	160	160	40		160	20				
5044	160	80	640	320	640	640		320	320	ran away Aug 91			
5045	1280	160	1280	640	320	320		1280	320				
5046	40	40	80	160	160	640		640	1280				
5047	320	160	160	320	80	20		1280	1280	1280	died Jun 91 non-effusive FCoVV		
5049	40	40	160	80	80	rehomed							
5050	160	80	1280	80	20	320		10	0				
5051	160	80	80	20	10	40		40	10				
5052	40	80	10	0	20	40		20	10				
5053	40	0	20	0	10	80		40	10				
5767		40	160	40	160	640	640	640	640				
5768			160	10	80	1280		20	40				
5049/3/2*	Jun 640	Jul 1280	Aug 1280	Oct 89 640	Jan 320	Jan 1280	Feb 90 640	died Mar 90	non-effusive FCoVV				
5046/4/1*	0	40	80	10	160		640	Jul 640	Nov 90 320		May 91 160		

* These are kittens. The ref. no. refers to the queen's ref., the no. of litter then the no. of kitten within the litter. These kittens were sold, the new owner kindly allowed testing to continue.

Except where otherwise stated, these cats were well in Oct 91.

successfully with treatment.

4.3.5 Re-exposure of the seropositive cat to FCoV

Forty-seven cats were selected whose antibody level fell by 4 two-fold dilutions (if over 320 to begin with) or 3 two-fold dilutions (if 160 or 80) and then rose again abruptly. Cats whose antibodies disappeared were not included because the intention was to examine seropositive animals which had been re-exposed to FCoV either from without or by recrudescence of latent virus in their own bodies. No doubt, other cats with more even levels of antibodies throughout the survey were also re-exposed to virus, but since there was no way of detecting these cats, they could not be counted. Only one of the 47 cats (cat 5047) succumbed to FCoVV. Thus it appears that ADE is not a major problem in naturally occurring FCoV infection.

4.3.6 Reinfection of seronegative households

Cats in 9 of 24 households which had become predominantly seronegative seroconverted again. The reason could be traced to the introduction of a new cat (houses 1682, 1693, 1779, 1782, 1790 and 3117), as an example, household 1790 is illustrated in table 4.6. Two of the cats from household 1775 had gone to an untested stud; in household 1690, there was a free-ranging pet cat (5267) which alone remained seropositive when the rest of the household was seronegative and whose titre rose shortly before the other cats seroconverted again. Household 1690 is shown in table 4.7. The seronegative cats in household 1774 were allowed to mix with the three remaining seropositive cats and two new seropositive cats were introduced. In household 1779, a cat, which had not directly contacted the new arrival, seroconverted, showing indirect transmission.

Table 4.6 Sequential IFA tests on cats in household 1790 in which FCoV was re-introduced by purchase of an infected kitten.

Cat ref. no.	Oct 87	Nov 87	Dec 87	Jan 88	Mar 88	Jun 88	Oct 88	Apr 89	Feb 90				
5624	320	320	1280	40		0	0	20	320				
5625	320	160	320	20	20	0	0	40					
5626	160	160	1280	80		0		320					
5627	160	160	320	80	160	80	10	80					
5628	80	40	80	20	80	0	0	40	10				
5629	40	40	20	20	20	20	0	160					
5630	80	40	160	20		(preg.)	0	0					
5631	80	20	160	20	80	0	0	320					
5632	0	0	0	0	0	(preg.)	0	320					
5633	0	0	0	0		0			320				
5634	1280	1280	died Nov 87, ocular signs										
5635	20	0	20	0	0	0	0	320					
5636	0	0	20	0	40	0	0	0					
5637	0	0	0	0	0	0	0	20					
5705/1/1	purchased Jan 89, tested 80, returned to breeder												

Except where otherwise stated, these cats were well in Sep 90.

Table 4.7 Sequential IFA tests on cats in household 1690 in which FCoV was re-introduced by cat 5267 which was the only free-ranging cat.

Cat ref. no.	May 88	Jul 88	Oct 88	Jun 89	Nov 89	Feb 90	May 90	Jun 90	Sep 90	Feb 91	Oct 91		
5267	320	80	80	160	40	160	20	40	0	0	>1280		
5266	320	40	20		0			>1280		40	>1280		
5270	10	10	0		0			640	80	0			
5263	0	10	0		0		320	160	80	20			
5265	640	80			0			>1280	640	10	160		
5268	80	20		0	0			>1280		80	>1280		
5262	20	20	0		0			160	80				
5264	160	40	0	0	0		80	320	1280	20	320		
5269	320	40	0		0			1280	640	0	0		
5113	acquired Dec 88		0	0				>1280	1280	0			
5271	died Apr 88 FCoVV confirmed												
5073	acquired Jun 89			0				640	80	0	80		

Except where otherwise stated, these cats were well in Oct 91.

4.3.7 Loss of FCoV antibodies

In 24 households 50% or more of the cats became seronegative by June 1991. The survey households are presented in table 4.8. In 9 of these households cats subsequently became seropositive again. Therefore at the end of the survey, in 57 households over 50% of cats were seropositive. Antibody loss occurred in periods ranging from 3months (households 1594 and 1690) to 35months (1684).

In the 24 households, initially 37 cats were seronegative and 145 (80%) seropositive. At the end, 132 were seronegative and 44 (25%) seropositive. To check whether this was because the seronegative households had been followed for longer and therefore had more time to lose their antibodies, the intervals in months between the first and last tests were added together for each of the groups and divided by the number of households. In seronegative households: 357m divided by 24 households = 14.9m (range 3-35m.) In seropositive households 995m divided by 53 households = 18.7m (range 5-41m). Thus, the seropositive households had, on average, been followed for slightly longer than the seronegative households but the difference was not significant.

To determine if the number of cats in the households affected the outcome, the average number of cats in the households in the 2 groups was calculated. It has to be borne in mind that not all of the cats in a household might be tested for reasons such as age of cat or pregnancy. However these factors were similar for both groups. In the households in which cats became seronegative there were 290 cats, giving 12.1 cats per house. In the households where cats remained seropositive there were 538 cats, giving 10.1 cats per house. There were more cats, on average, in the households which became seronegative, though not significantly more. However, as is shown below, the grouping of the cats within these households was an important factor in the outcome.

Table 4.8 The outcome of the survey households.
The households are grouped under the reason for first presenting for FCoV testing. In 24 households, over 50% of cats became seronegative. The time from first testing to most being seronegative is given along with the time the household was surveyed. The bracketed households became negative then IFA titres rose again.

Owner ref	start +	start -	No. new cats	end +	end -	Inter -val	Time total	No. FCoVV	Re- home	Dead other
Cats died of FCoVV on premises										
216	8	0	-	4	4	4m	7m	0	0	0
1594	6	1	1	2	5	3m	3m	0	1	0
1596	10	0	-	6	3xU ?	8m		0	0	0
1613	6	0	2	1	3	24m	36m	0	0	2
1615	22	1	1	18	2	17m		2CA	4	0
1627	3	2	-	3	1	5m		0	0	0
1673	5	3	-	2	5	5m	5m	0	0	0
1674	6	3	-	0	8	11m	23m	0	0	0
1677	24	1	1	20	4	26m		2CA	0	6
1678	10	0	2	8	3	20m		0	0	1
1680	6	0	6	11	0	31m		0	0	1
1682 }	6	0	1	1	4	14m		0	0	0
}				4	2		39m	0	0	0
1683	11	2	-	11	2	5m		0	0	0
1690 }	9	2	-	1	10	3m		0	1	0
}			2	11	1		29m	0	0	0
1692	5	3	-	2(2x 10)	6	8m		0	0	0
1693	6	7	2	0	12	9m	30m	0	2	0
1694	2	1	-	0	2	9m	9m	1SA	0	0
1768	14	0	-	12	0	14m		1CA 1CK 1SK	0	1
1769	2	0	1	1	2	30m	30m	0	0	1
1770	14	0	-	13	0	4m		1S	0	1
1773	4	5	-	9	0	11m		0	0	1
1777	16	4	4	5	9	9m			3	1
			4	13	4		41m	1	9	3
1778	8	1	-	2	7	14m	14m	0	0	0
1781	8	1	3	8	0	12m		1SA	1	1
1784	4	0	-	1	3	6m	6m	0	0	0
1785	4	0	-	3	1	32m		0	0	0
1790 }	10	4	2	2	12	8m		1SA	0	0
}				12	3		19m			
2097	1	0	-	1	0	28m		0	0	0
3122	21	3	3	16	5	6m		0	6	0
3123	8	4	1	6	2	9m		0	2	2
3124	4	0	1	3	2	19m		0	0	0
Sold kittens died of FCoVV										
1672	6	0	1	3	0	17m		2SA	2	0
1675	6	0	-	4	2	25m		0	0	0
1684	3	1	2	2	3	35m	35m	1CA	0	1
1686	5	0	-	3	2	5m		0	0	0
1691	7	0	-	6	0	9m		0	1	1
1771	5	0	1	3	3	26m	26m	0	0	0
1775 }	2	1	-	0	4	4m				
}			1	4	0		34m	0	0	1
1776	5	0	3	6	0	34m		1CA	1	0

Table 4.8 contd.

Owner ref	start +	start -	No. new cats	end +	end -	Interval	Time total	No. FCoVV	Rehome	Dead other
1779	3	0	2	0	5	15m	28m	0	0	0
1789	24	1	6	29	2	26m		2CA 1SA	0	2
1801	22	1	-	15	3	17m		0	3	0
3117 }	10	5	1	6	6	5m				1
}			3	9	2		21m	0	5	2
3121	8	1	-	7	1	24m		0	3	0
3125	5	0	-	4	0	16m		0	0	0
Clinical signs suspicious of FCoV infection										
54	7	0	1	4	0	14m		0	4	0
1685	41	1	3	31	6	31m		1C 1S	5	10
1772	5	0	-	2	3	25m	25m	0	0	0
3095	6	1	1	7	1	14m		1 CK	0	0
Clinical history indicative of FECV infection										
1598	17	0	4	19	1	31m		2C	2	1
1612	2	0	-	2	0	6m		0	0	0
1676	14	0	1	14	1	29m		1CK	1	1
1780	4	0	-	3	1	12m		0	0	4
In contact with real or suspected FCoV excretor										
1614	9	0	-	9	0	5m		0	0	0
1681	8	0	-	4	0	8m	8m	0	6	1
1687	13	0	-	11	0	30m		0	2	0
1689	10	0	-	7	3	26m		1	0	1
1774 }	10	2	2	4	10	11m				
}			5	5	9		29m	0	4	1
1788	13	2		8 (2U)	3	24m		0	2	3
2439	1	0	-	1	0	9m		0	0	0
2863	7	0	1	5	2	24m		0	1	0
No history indicative of FCoV infection										
1595	7	3	1	10	0	37m		0	1	2
1679	6	3	-	3	5	22m	40m	0	1	2
1695	10	0	-	10	0	7m		1CA	0	0
1782 }	8	0	-	4(3x10)	4	16m		0	0	0
}			3	11	1		32m	0	0	0
1786	4	1	-	2	3	26m	37m	0	0	0
3119	1	2	-	3	0	21m		0	0	0
3120	6	3	1	7	1	28m		0	2	1
Reason for first sampling unknown										
3	2	2xU	-	4	0	7m		0	0	1
1688	2	2	-	3	1	10m		0	0	0
1783	19	0	-	19	0	12m		0	6	1
1787	9	3	-	11	1	24m		1S	0	0

S = suspected of FCoVV
C = histopathologically confirmed
A = adult
K = kitten

\+ = positive
\- = negative

The next factor to be examined was the antibody titres in each of the households. Presumably, cats which mount a high antibody response take longer to become seronegative than those which have less antibodies. In 22 households the highest titre reached was less than or equal to 640, while in all of the other 50 households one or more cats had had an IFA titre of 1280. Eleven of these 22 households went on to become mostly seronegative, which is obviously a greater proportion than in those households which remained seropositive.

In the households which became seronegative at the end, 37 cats were seronegative at the beginning and 145 were seropositive. In those households which remained seropositive throughout the survey, 473 were seropositive at the start and 45 were seronegative. This is a very significant difference by chi-squared analysis ($p < 0.001$). Therefore the proportion of seronegative cats at the end is influenced by the proportion of seronegative cats at the start. This may seem obvious, but it should be noted that it was not necessarily the same cats which remained seronegative throughout the survey (as can be seen in table 4.7): many of those which began the survey seronegative became seropositive and vice versa.

A computer programme was devised to select cats whose IFA titres had fallen from 320 or over to 20 or less, and those whose titres had risen from less than 20 to 320 or over by June 1990. The IFA titres of 71 cats had risen, of which one had died of FCoVV. The antibody titres of 96 cats had fallen, and one of these cats had died of FCoVV.

The groupings of the cats were classifed as 'small' if up to 3 cats, 'medium' if 4-10 cats and 'large' if over 10 were present. Where the groupings of the cat altered, the predominant grouping around the time of the titre change of interest was taken. The titres of some cats rose and fell. Of those whose titres had fallen, 20 were housed in small groups, 30 in medium and 18 in large and 3 were unknown. Of those

whose titres had risen, 44 were in small groups, 25 in medium, 20 in large and 7 unknown. Group size was found to significantly affect whether a cat's titre rose or fell (Chi^2 = 6.88, $p < 0.05$ at 2 degrees of freedom). Neither the cat's age nor whether pedigree or domestic affected the titre.

A similar programme selected cats whose antibody titres remained at less than or equal to 20 for 3 or more tests. One hundred and forty-four cats had mainly low titres for an average of 66.2 weeks (and 3 for an unknown time): of these 55 were in small groups, 48 in medium groups, 29 in large groups and the group size of 14 was unknown. One cat of 144 died of FCoVV.

Fifty cats had IFA titres equal to and over 640 for at least 3 tests and there were 4 deaths to FCoVV in this group. Of these, 16 cats were in small groups, 22 in medium groups, 8 in large groups and the group size of 4 was unknown. Chi^2 was 2.73 which is significant ($p < 0.25$) at 2 degrees of freedom.

4.3.8 FCoVV in FeLV and FIV infected households

Seven out of 43 households had endemic FeLV infection and 6 of 29 households had FIV positive cats. Three cats from FeLV positive households and 14 from FeLV negative households developed FCoVV. Comparison of cat mortality due to FCoVV in houses which had FeLV and those which did not, showed that there was no significantly greater mortality rate in houses in which FeLV is endemic. Cats from 4 FIV positive and 8 FIV negative households died of FCoVV which is significant ($p < 0.25$).

4.4 DISCUSSION

4.4.1 FECV or FIPV?

In the literature, much has been made of the range of pathogenicity of FCoV which can be found in cats inoculated with laboratory strains [9, 11, 23, 66, 96, 104, 111, 113, 133, 126]. Veterinarians have been led to believe that the presence of FCoV antibodies in a cattery may not necessarily indicate the presence of a virus capable of causing FCoVV. In this survey, there were FCoVV deaths in all the groups of seropositive households, regardless of the first reason for presentation. Therefore an important finding in this study was that no distinction can be made between strains of FCoV in the field on the basis of clinical and serological history. All FCoV infections should be regarded as potentially pathogenic.

4.4.2 The mortality rate in seropositive households

The mortality rate was highest (12%) at the start of the survey in those households which were included because they had had a death from FCoVV. With time, the mortality rate fell in this group to the same level as found in seropositive cats in all the other groups (3-4%). This finding agrees with previous authors who reported that morbidity due to FCoVV rarely exceeds 30% in cattery reared cats and in most cases is less than 5% [102, 105]. An explanation for this finding may be that the cats in households which presented for FCoVV death were meeting the infection for the first time and therefore had no immunity to it.

Whether or not the outcome of FCoV infection in any particular cat depends on the strain of the FCoV or on the cat's immune response to the infection remains to be seen. Certainly, no household sustained large losses from which one might extrapolate that there was no particularly virulent strain of FCoV at large in these households. Death rates fell after what is presumed to be the primary infection indicating that some degree of immunity or resistance to infection existed in most

of the cats.

The figure of 3-4% mortality may well rise as the remaining cats are followed over the next few years because deaths from seemingly unrelated causes (kidney failure and neurological signs) were shown to have their origin in FCoV infection. Since cats 5038, 5047, 5149 and 5684 had incubation periods of 3 years or more, it is also likely that there will be more losses due to classical FCoVV as we continue to observe the cats. Presently there is no way of distinguishing an immune cat from one which will succumb to FCoVV.

4.4.3 IFA titres in cats which developed FCoVV

In experimental infections of cats with FCoV, the antibody titre rose rapidly and consistently and this was assumed to be the pattern which would be found in the field [49, 71, 102, 112, 140]. The results of this survey show that this is not the case. While 9 cats which developed FCoVV did have steadily climbing IFA titres and 4 had consistently high IFA titres, in 5 other cats, IFA titres actually fell. In cats 5040, 5153, 5164, 5340 and 5508 the IFA titres never rose above 320, or indeed 40 in the case of cat 5040. One explanation of this finding is that it is not the titre of antibodies that is important, but whether the balance of antibody and virus is such that ICs can be formed. Thus a cat which has a low antibody response to FCoV will still be in danger if the amount of virus in its blood is such that ICs can be formed and vascular damage ensue.

Interestingly, none of the 48 cats which remained IFA negative throughout the survey developed FCoVV although, as is shown in 6.3.5, many had anti-N antibody and must have been exposed to virus.

Cat 5569 was seropositive from March 1988 to April 1991 and her IFA titre was 320 for two years falling to 40 latterly. She did not shed virus because, first, all 12 kittens born into

the household were in contact with her and were seronegative and, secondly, all antibody titres of all the other adults fell to zero.

4.4.4 ADE in the field

ADE of FCoV infection is a commonly reported phenomenon which has foiled many vaccine attempts [51, 83, 107, 110, 111, 112, 114, 149]. In this survey, previously exposed (i.e. seropositive) cats in the field did not appear to be more susceptible to FCoVV. Indeed, the mortality rate in households which had already lost a cat to FCoVV appeared to be less than at the time of index case presentation.

An explanation of the discrepancy between laboratory results and field observations may be that, in the former conditions, virus dose is possibly much greater than in nature. Experimentally, sublethal amounts of virulent virus have been shown to be protective but very high doses were almost always lethal [112]. Secondly, many experimenters administer the virus parenterally, bypassing the mucosae which are the cat's first line of defence against this infection. Difficulty in infecting cats with FCoV orally has been reported [49, 71]. In an experimental attempt to mimic natural infection, up to 6 virus exposures and finally subcutaneous inoculation were needed to induce seroconversion and disease in a group of cats [71].

It is possible that in the majority of FCoV infections the virus remains in the alimentary tract and never becomes systemic. Like TGEV and CCV, it is primarily an intestinal pathogen.

4.4.5 Loss of FCoV antibodies

One third of the survey households became mainly seronegative and seemed to have eliminated the infection. Fall of IFA titres from over 320 to 20 or less was found to be significantly associated with keeping the cats in groups of 3

or less. In Chapter 5 it will be shown that approximately one in 3 seropositive cats excretes virus, therefore it seems likely that this management system simply reduces exposure to virus. Cats which were kept in larger groups remained seropositive indefinitely. Cats could remain seropositive for a long time (37 months or over) without exposure to external virus as was shown by cat 5569 which was in a household into which several litters of seronegative kittens were born. Her IFA titre remained about 320 for over 2 years then gradually fell to 40 while the other adults in the household became seronegative.

A study of sequential IFA titres in naturally occurring FCoV infection has only been recorded once before in a colony of 32 cheetahs, of which some still had antibodies after 4 years [16].

4.4.6 Reinfection of seronegative households

No cat which became seronegative seroconverted again without a history of exposure to a possible external source of virus. Therefore seronegative cats do not appear to harbour latent virus which can recrudesce.

4.4.7 Conclusion

The question facing veterinarians when FCoV is endemic in a household is whether or not IFA testing is worthwhile. The answer to this question will depend on the reason why testing is being considered. If it is to try to establish the virus excreting status or prognosis of a particular cat within the household, then testing the adults will not be useful. Neither will a one-off test of a colony be particularly helpful unless it is to establish whether or not FCoV is endemic within the colony. However, repeated testing will determine whether or not the household is the one in three which will become seronegative and therefore the virus excreting status of the whole household will be determined.

Repeat testing is useful for cat owners who are willing to keep

seropositive and seronegative cats apart in small groups in order to help their household to become seronegative. However, as is shown in Chapter 5, the cat with an antibody titre of 40 is just as likely to excrete virus as one with a titre of 1280 so grouping the seropositives according to titre is probably not useful.

Testing is best performed every 3-6 months in the field, as significant change in IFA titre in less than 3 months is uncommon.

CHAPTER 5

A STUDY OF KITTENS FROM QUEENS NATURALLY EXPOSED TO FCoV

5.1 INTRODUCTION

5.1.1 Source of infection

In the UK, the highest incidence of FCoVV is in pedigree cats up to eighteen months of age (fig.s 3.8, 3.9) [140]. Given the lack of a history of contact with other cats in their new homes, it seemed reasonable to assume that most of these cats became infected in the cattery of origin rather than by meeting the virus after sale [114]. As stated in Chapter 4, many of the survey households only became aware of the presence of FCoV in their establishments when a kitten which they had sold went on to die of FCoVV (table 4.1).

In this study, we were interested to discover what happened to kittens born into seropositive households. The serology of these kittens was investigated in conjunction with other factors such as age, breed of queen and management of kittens. The serology of naturally infected kittens has previously only been examined in one household [110].

FCoV appears to be spread mainly from carrier queens to their kittens within the first few weeks of life [114]. Virus is probably ingested after birth [106] although there is evidence of transplacental [85, 106] and indirect transmission [110]. However, the source of infection has remained speculative [9]. Since the virus cannot survive for long periods outside the host, indirect transmission is unlikely to be a major source [100]. Most cats which develop FCoVV have no history of being in contact with a clinical case of FCoVV. It has been postulated that FIPV could be a virulent mutant of a chronic, asymptomatic FECV infection [110, 108]. The most likely source of FCoV is healthy, carrier cats [106].

The existence of healthy seropositive carriers has been demonstrated by placing 12 week old SPF kittens with healthy seropositive cats. Seroconversion of the kittens occurred after 2 - 10 weeks [110].

Many questions remain about FCoV carrier cats. For example, it is not known if carrier cats go on to develop FCoVV; how long they excrete virus; whether seronegative cats excrete virus; if any particular antibody titre is associated with virus excretion; or what proportion of seropositive cats are carriers [9]. Those questions were addressed in this study.

Another question which was considered was at what age should kittens be tested to gain an accurate idea of whether or not they had been infected.

5.1.2 Health of the kittens

Given the long incubation period of FCoV from time of infection to development of clinical signs, health was monitored before and after sale of the kittens as far as possible.

Circumstantial evidence suggests that initial infection with FCoV is accompanied by mild URT signs [5, 132, 140] or diarrhoea [132,140]. There are many possible causes of diarrhoea in kittens apart from FCoV: dietary, parasitic, bacterial and viral. Therefore the prevalence of antibodies to FCoV was examined in kittens with and without diarrhoea. Kittens in both groups were from the same households so that other environmental factors would be the same as far as possible. FECV is alleged to cause inapparent to mildly severe enteritis in kittens between 5-12 wks of age [103, 110, 111]. Enteritis has also been reported in field cases of FIP [48].

In experimental infections, pre-existing antibody enhances the disease [109, 110, 112, 156]. It has also been suggested that fewer cats develop FIP after FIPV ingestion if they had been

previously infected with FECV but that in those which do develop the disease it is enhanced [111]. However, MDA may confer some degree of immunity; eight kittens born to immune queens were protected from challenge at 8-10 weeks of age [114]. In the present study, the health of the kittens after they left their breeding establishments was monitored to try to determine if seropositive kittens were more of less likely to develop FCoVV.

5.1.3 Kitten Mortality Complex (KMC)

KMC is defined as reproductive failure (failure to concieve; foetal resorption; abortion; stillbirths; congenital birth defects) - kitten mortality (fading kittens; acute congestive cardiomyopathy: FIP) [125]. FCoV has been implicated in the KMC [57, 85, 89, 99, 125, 153].

While there is no doubt that there is a high incidence of FCoV seropositivity in many catteries which have this complex, it is also true that it has been observed in seronegative catteries and that there are seropositive catteries in which it does not exist [104, 114].

In this survey, the incidence of the various signs which make up this complex was examined.

5.2 MATERIALS AND METHODS

5.2.1 Source of infection

Kittens from the survey described in Chapter 4 were bled one to three times before they were sold. Only kittens over 7 weeks and under 16 weeks of age are included in table 5.1. Their antibody titre was measured by IFA as described in Chapter 2. Low antibody titres are difficult to interpret, so whenever there was a kitten with a titre of 10 the serology of its littermates was taken into consideration. When several of a litter were seronegative but one kitten had a titre of 10, that litter and kitten were considered to be seronegative.

Four hundred kittens from 98 queens fitting the criteria above were sampled. Fifteen queens had more than one litter. The cats were from 41 households, 19 of which had cases of FCoVV in adults and 12 had cases in kittens which had been sold out of the house. Four had been in direct contact with cats in households which had had a case of FCoVV. In 2 households testing was initiated because the cats had chronic diarrhoea. FECV had been diagnosed histologically in one and was suspected in the other. In 4 households there was no history of FCoVV or diarrhoea.

Kittens were classified according to their environment at the time of the first blood sampling. These were: 'normal' (N) if the kittens were allowed to mix with the rest of the household, 'mother' (M) if the kittens were isolated with their mother and 'isolated' (I) if the kittens were isolated from all adult cats, including their mother, from 2 - 6 weeks of age. Choice of kitten environment was entirely at the breeder's discretion.

The reference numbers of the kittens refer first to the queen's reference number, the number of the litter (which is an indication of how many litters that queen had had) then the number of the kitten within that litter.

Table 5.1 Effect of environment on FCoV infection of kittens from seropositive households

	Environment		
	Normal	Mother	Isolated
Total no. of litters	81	39	12
Total no. of kittens	238	114	46
Litters becoming seropositive			
No. of litters	50	11	0
Total no. of kittens	134	39	0
No. seropositive kittens	124 (52%)*	35 (30%)	0
No. developing FCoVV	5**	1***	0
No. seronegative kittens	10 (4%)****	4 (4%)	0
No. developing FCoVV	0	0	0
Litters becoming seronegative			
No. of litters	31	28	12
No. seronegative kittens	104 (44%)	75 (66%)	46 (100%)
No. developing FCoVV	0	0	0

Key:
No. = number
FCoVV = feline coronavirus-associated vasculitis

* percentage of total no. of kittens in that environment

** 2 diagnosed by clinical signs and titre, 3 confirmed by histopathology.

*** confirmed by histology

**** In one of the litters all of the kittens were seronegative at 9 weeks and one kitten seroconverted at 16 weeks. The titre of the other two kittens was not available so these two are counted neither positive nor negative.

5.2.2 Health of the kittens

Breeders were requested to report on the kitten's health prior to sale. They were asked if there had been any diarrhoea; failure to gain weight as normal; sneezing; ocular discharge; or other signs. The breeders were contacted regularly to ask if they had heard how the kittens were doing in their new homes. Unfortunately, few of the people who bought the kittens allowed sampling after the kittens had left the breeding establishment. Some kittens were kept by their breeders and became part of the general survey.

I am grateful to Dr Chris Robertson for statistical analysis of the kittens' health data.

5.2.3 Kitten Mortality Complex

Post mortem material was specifically requested from abortions, stillbirths, fading or deformed kittens from seropositive households for histopathology. Because seropositivity does not necessarily reflect infectivity, the incidence of these abnormalities was examined in litters where presence or absence of FCoV antibodies in littermates was known.

5.3 RESULTS

5.3.1 Waning of MDA and seroconversion of kittens

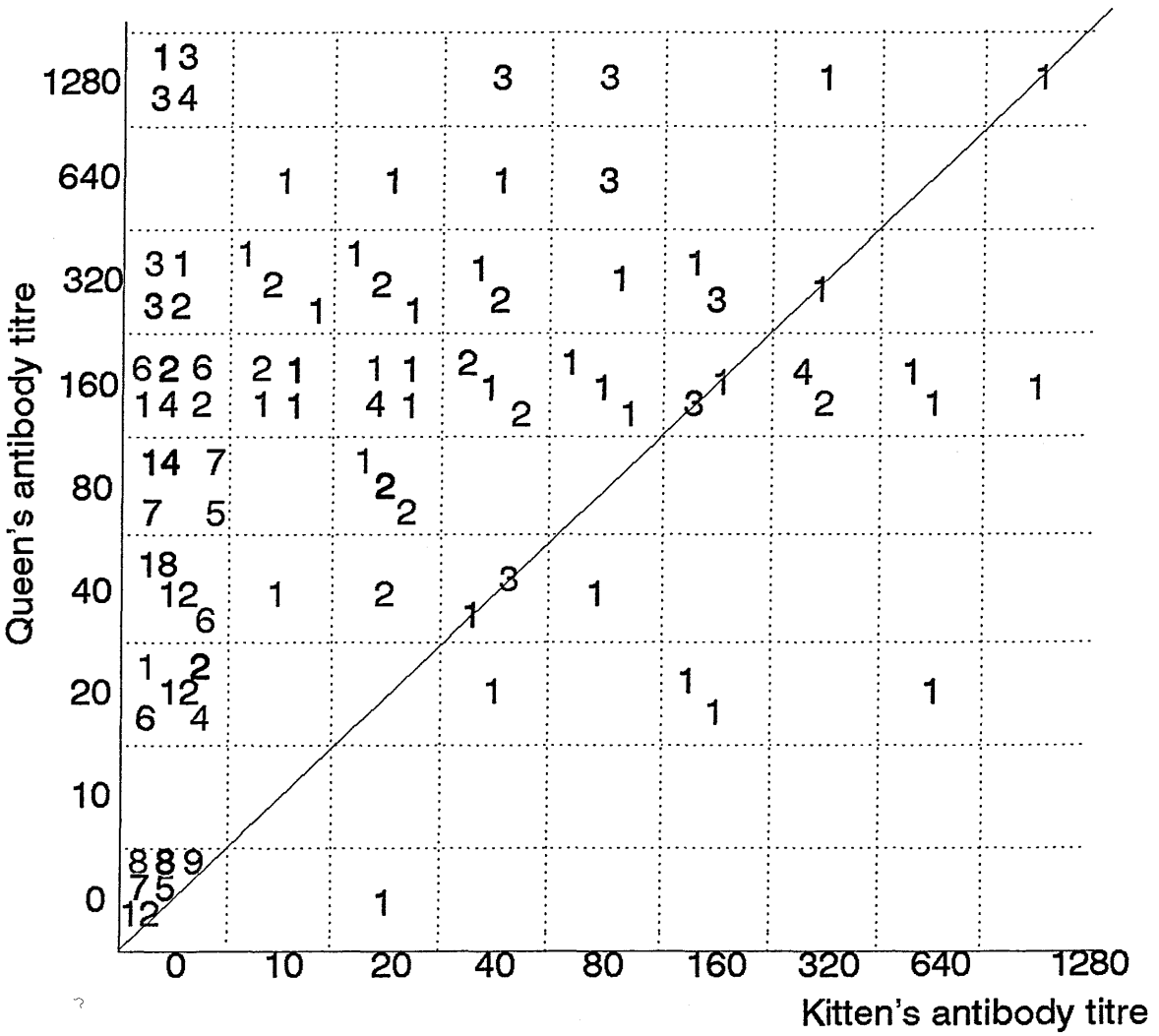
It was assumed that maternal antibody to FCoV disappeared by 7-8 weeks of age [111, 114] and that any antibody titres found in older kittens thereafter truly reflected exposure of the kittens to coronavirus. This assumption seemed reasonable since five queens with high titres (1280) in M and I environments produced 15 kittens which had no detectable antibodies at eight, nine, ten and twelve weeks respectively at the time of first testing. Litter 5691/2 had antibodies at 6 weeks which had gone by 9 weeks.

Sequential weekly testing of kittens from birth has only been reported once [111] where MDA was found to wane by 4-6 weeks of age. Antibody reappeared around 8-14 wks of age as the kittens became infected. In this survey, plotting the titre of the kitten against that of the queen supported this time scale since kitten antibody levels equal to, or greater than, those of the queens started to appear from 8 wks (Fig. 5.1).

Fig. 5.2 shows the proportion of seropositive and seronegative kittens aged from 1 to 12 weeks. The percentage of seronegative kittens falls between 5 and 10 weeks and the percentage of seropositive kittens rises. After ten weeks the levels remain around 50%. It appears that not all infected kittens are able to seroconvert before 10 weeks of age.

In only a few litters were 3 blood samples available for more than one member of the litter. In litter 5717/1 two out of three kittens were seronegative at 8 weeks, but all 3 had become seropositive by 12 weeks. Litter 5043/1 was seronegative at 10 weeks and seropositive at 14 weeks. Three of 4 kittens of litter 5760/1 were seropositive at 8 weeks and all were seropositive by 11 weeks. Litter 5476/2 was seronegative at 9 wks though 5476/2/2 was seropositive at 15 and went on to die of FCoVV. In the same household only 2 of 6

Fig 5.1



Age of seroconversion of kittens.

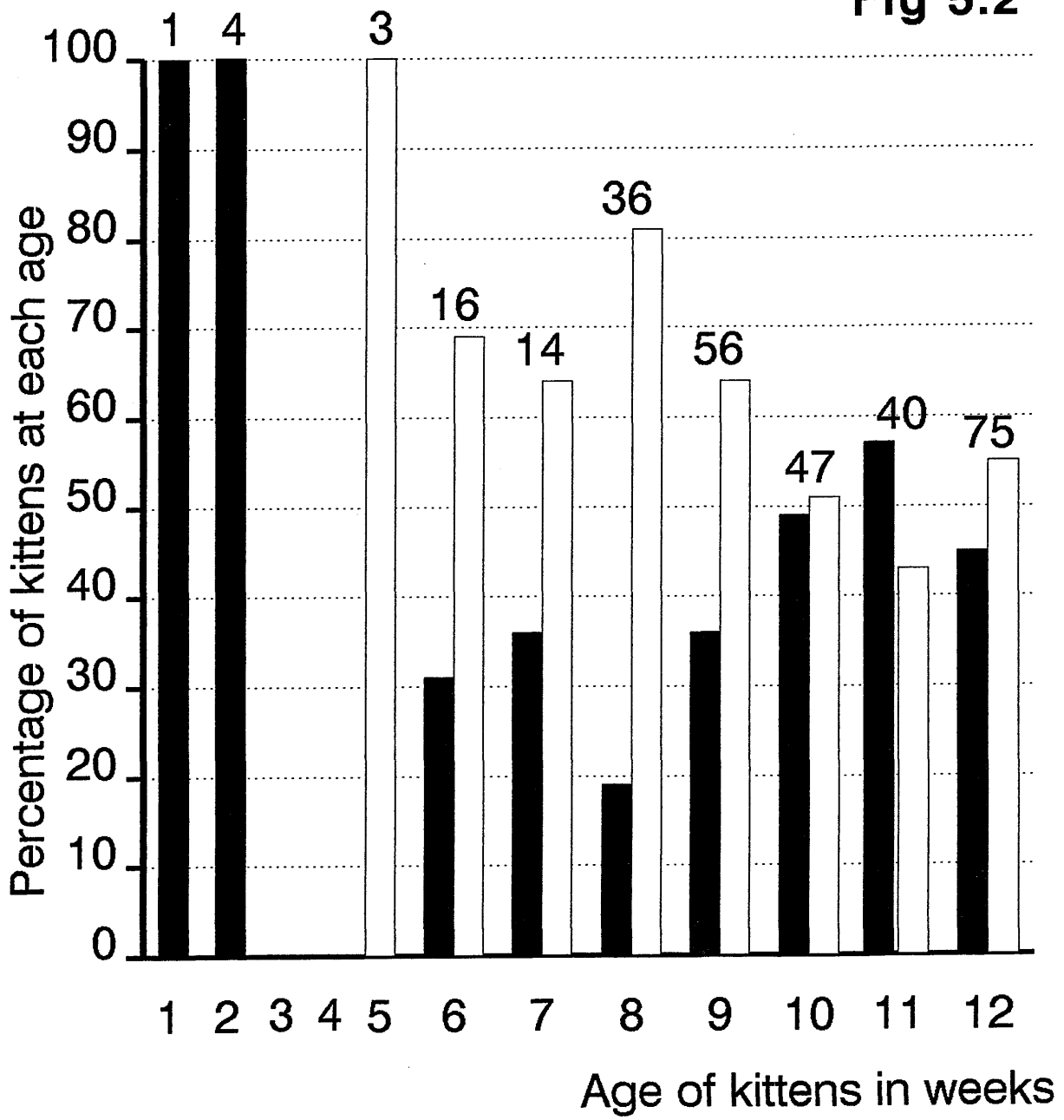
The numbers indicate the number of kittens at each age.

To the right of the diagonal, kittens' titres are greater than those of their mothers, therefore they must be producing their own antibodies.

Key:

6 Weeks	9 Weeks
7 Weeks	10 Weeks
8 Weeks	11 Weeks

Fig 5.2



The proportion of kittens which have seroconverted at each age. The total number of kittens of each age is given at the top of the column.

Key:

Seropositive

Seronegative

in litter 5473/1 were seropositive at 12 weeks.

The kittens of litter 5340/2 were all seropositive at 9 weeks with titres of 20, 20, 80, 40 and 10 respectively. Kittens 5340/2/1, 4 and 5 were seronegative by 13 weeks and 5340/2/2 and 3, whose titres were 20 and 80 at 9 weeks, had titres of 20 and 40 by 13 weeks. Kitten 5340/2/3 went on to die of FCoVV. The queen's titre at the kitten's birth was 40 and had risen to 320 when the kittens were tested at 9 weeks of age.

5.3.2 Influence of environment

The result of the effect of environment on infection of kittens is shown in Table 5.1. Clearly the management of the seropositive queen and her kittens strongly influenced the prevalence of exposure to FCoV. The differences in proportion of seropositive kittens in each environment were highly significant by Chi-squared analysis ($p < 0.05$).

Table 5.2 shows the seroconversion and environment of litters from queens which had more than one litter. This confirms that kittens kept in a N environment were more likely to seroconvert. Queens which had had a seronegative litter in I or M environment had a seropositive litter in the N environment (queens 5047, 5265, 5444, 5633, 5695 and 5698) despite their own titres being zero or falling.

Figs 5.3, 5.4 and 5.5 show that the age, breed and antibody titre of the queens were evenly distributed throughout the three environments.

5.3.3 Virus excretion by queens

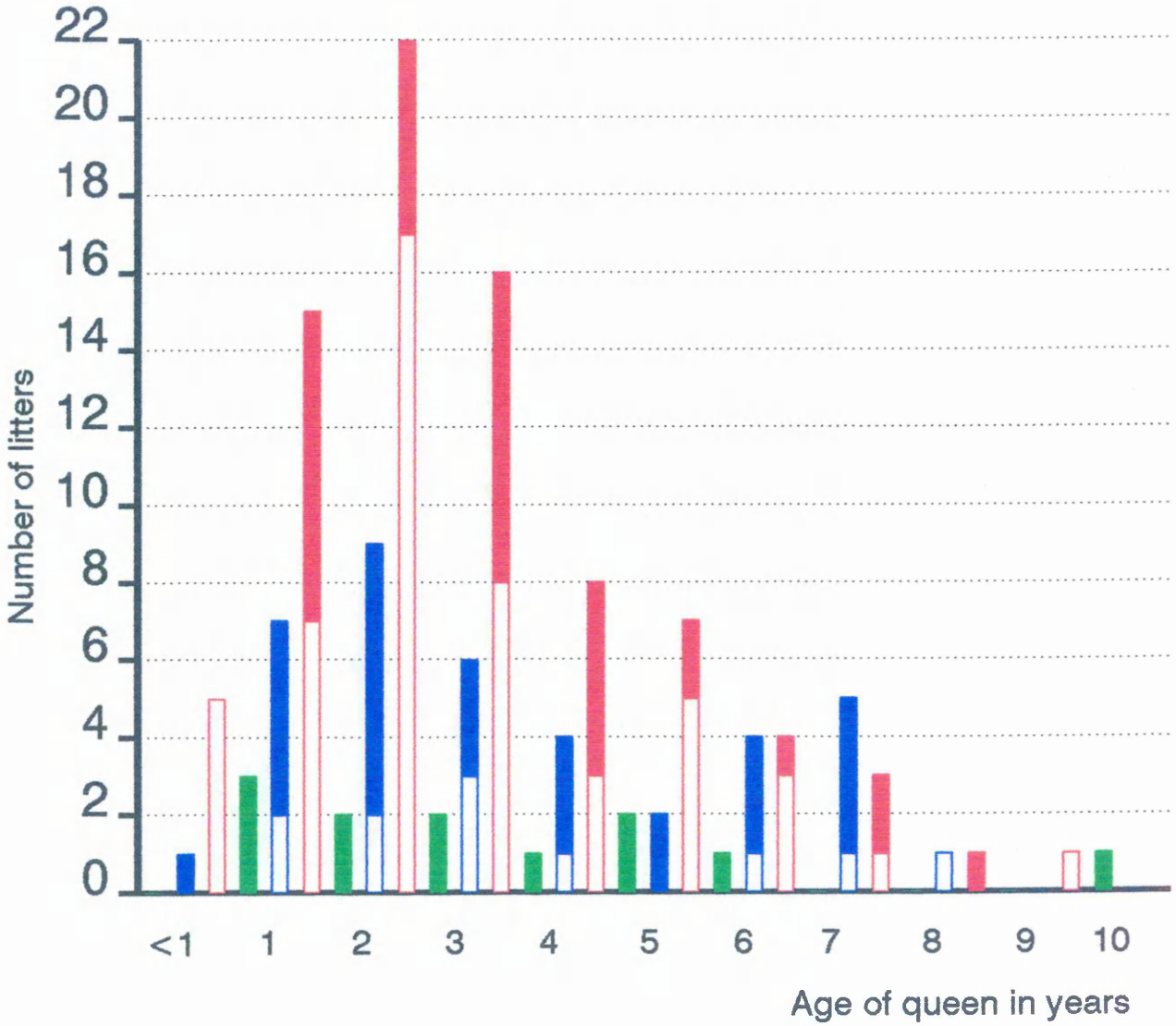
Twenty-seven kittens in 10 litters from 10 seronegative queens in the M environment were seronegative at 7,8,9,10,12 and 14 weeks. Five litters were born at a time when the antibody levels in the households had fallen or were falling to zero. One litter of 8 kittens from a seronegative queen were all seronegative at 6 weeks of age and two which were retested

Table 5.2 Seroconversion of successive litters from the same queen

Queen ref.	Litter no.	Envir.	Kittens seropos/neg	Queen's titre
5015	2	M	+	160
5015	3	N	+	1280
5043	1	M	+	160
5043	2	N	+	40
5047	1	M	-	80
5047	2	N	+	20
5111	1	N	+	80
5111	2	M	+	320
5144	3	N	+	160
5144	4	M	-	1280
5265	3	I	-	80
5265	4	M	+	1280
5268	2	M	-	80
5268	3	I	-	20
5340	2	N	+	320
5340	3	M	+	320
5444	7	I	-	40
5444	8	M	-	0
5444	9	N	-	0
5444	10	N	+	40
5460	2	N	-	40
5460	3	I	-	1280
5476	2	N	+	0
5476	3	I	-	0
5633	2	M	-	0
5633	3	N	+	0
5695	1	M	-	0
5695	2	N	+	0
5696	1	N	+	0
5696	2	M	-	40
5698	1	M	-	0
5698	2	N	+	1280
5698	3	M	-	320

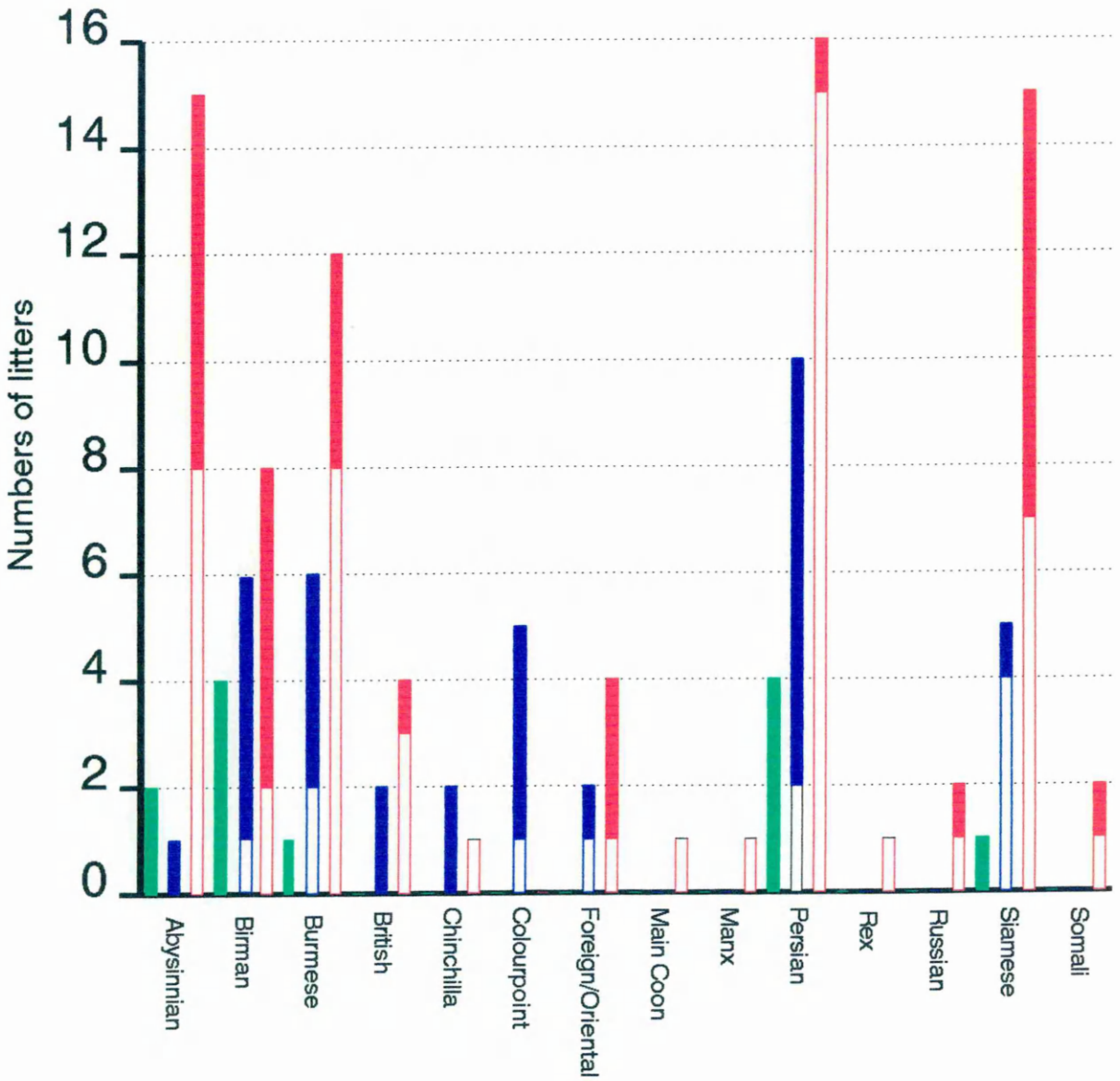
Key:
+ = seropositive
- = seronegative

Fig 5.3



The even distribution of queens' ages throughout the three environments shows that seroconversion of the kittens was unrelated to their mother's age.

Fig. 5.4

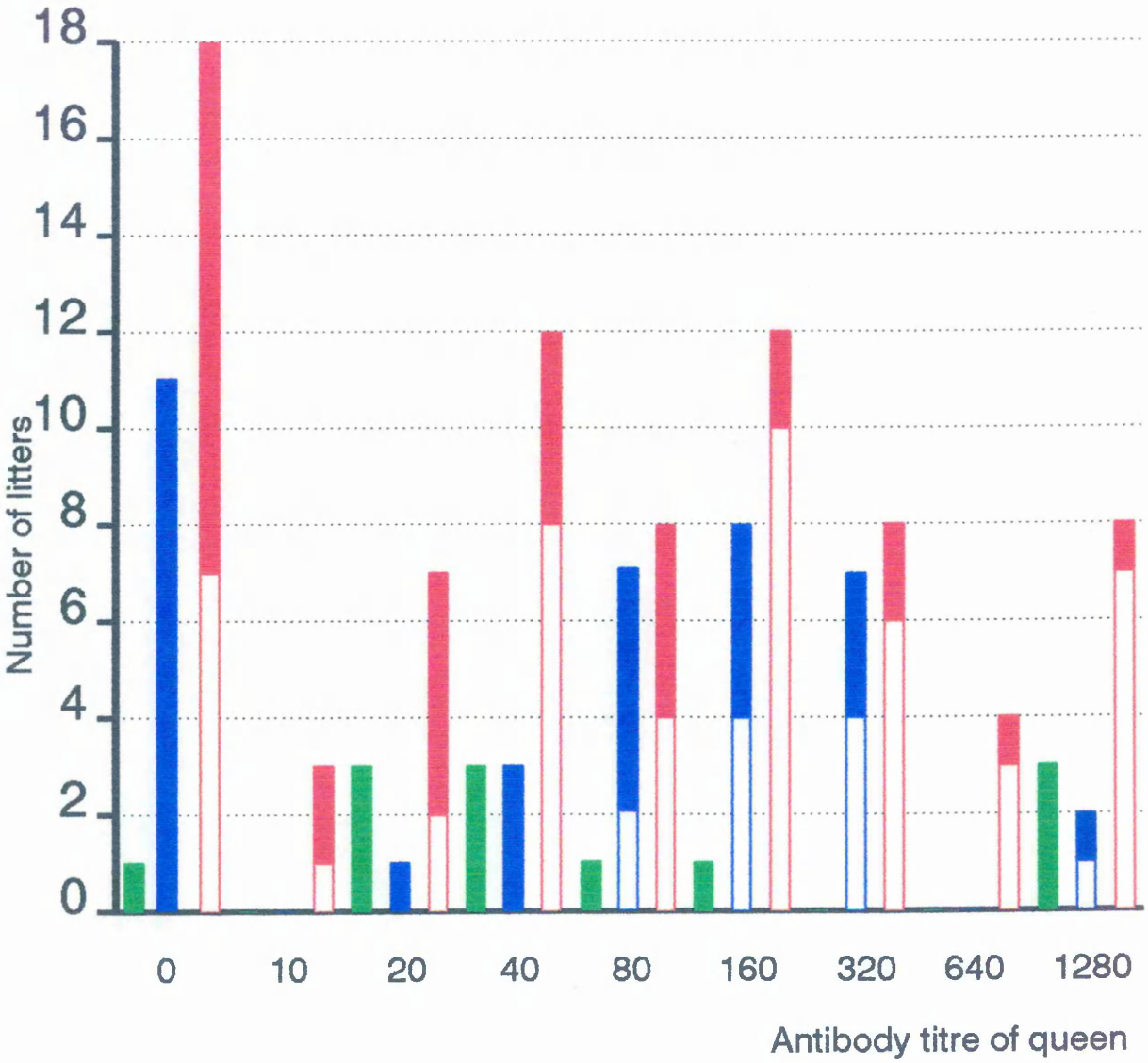


The distribution of breeds between the litters in each of the three environments, showing whether each litter seroconverted.

Key:

- Seropositive
- Seronegative
- Isolated
- Mother
- Normal

Fig 5.5



The distribution of antibody titres of the queens in the three environments, showing that the antibody status of litters was not related to the queen's titre.

Key:

Seropositive

Seronegative

Isolated

Mother

Normal

remained seronegative at 15 weeks. There were no seropositive kittens from seronegative queens in the M environment.

Serial blood samples and information on the health of the queens in the M environment were available for 10 out of 11 queens which had had a litter of seropositive kittens. These are presented in table 5.3. Eight queens were still well on average 16.5 months after giving birth to the seropositive litter (range 2-30 months.) One died of histopathologically confirmed effusive FCoVV 5 months later; one was put to sleep because she had had a seropositive litter.

5.3.4. Health of the kittens

Table 5.4 shows the history of those kittens which died of FCoVV. There are more kittens recorded in this table than shown in table 4.1 because this table includes those cats which were excluded previously because of age or lack of known antibody status. From table 5.4 it can be seen that no particular antibody titre, nor any pattern of titres, can be said to be predictive of the development of FCoVV.

The range of ages of death due to FCoVV was 1.8-53.2 weeks, though most deaths occurred before 35 weeks of age. It seems reasonable to suppose, therefore, that the majority of kittens which are still well a year after first being tested will remain well.

Table 5.5 shows the fate of kittens in the three different environments. No kittens which were seronegative went on to die of FCoVV.

Of the seropositive kittens in the N environment which were retested after 16 weeks, 1 was seronegative by 19 weeks; the 2 littermates of one which died of FCoVV were still seropositive at 18 and 25 weeks and still well at 55.4 weeks of age; 1 was still seropositive at 17 and 24 weeks; another at 19 and 26 weeks; 1 at 20 and 27 weeks; 1 still seropositive at 20 weeks

Table 5.3 Antibody titres and fate of virus excreting queens in the Mother environment

Queen's ref.								Queen's fate
	Oct	Mar	**Jan**[+]	Jun	Oct	Jun		Aug
5015	87	88	**89**	89	89	90		90
	0	0	160	10	0	1280		Fine
	Apr	Oct	Jun	Oct	**Jan**	Jun		Sep
5043	88	88	89	89	**90**	90		90
	40	80	80	160	160	40		Fine
	Feb	Mar	Jul	Sep	Mar	May	**Oct**	Sep
5111	88	88	88	88	89	89	**89**	90
	40	20	40	40	80	80	320	Fine
	May	Jul	Nov	**Jun**	Aug			Jul
5265	88	88	89	**90**	90			90
	640	80	0	1280	640			Fine
	Mar	Jun	Jun	**May**	Aug			Aug
5340	88	88	88	**89**	89			89
	40	320	40	320	160			Wet FCoVV confirmed*
	Aug	Oct	Jan	Apr	**Sep**	Apr	Oct	Oct
5419	88	88	89	89	**89**	90	90	90
	320	80	160	160	320	20	0	Fine
	Apr	Jun	Mar					Sep
5587	**88**	88	89					90
	80	80	0					Fine
	Feb	Jun	Oct	Feb				Oct
5717	**89**	89	89	90				90
	160	40	20	0				Fine
	Feb	May	Oct	Jan				Oct
5718	**89**	89	89	90				90
	160	40	320	320				Fine
	Oct	Jan						Mar
5746	**88**	89						89
	80	80						euthanased at owner's request

+ Dates in bold are tests nearest the time of the litter in the M. environment

* 5340/1 was three stillborn kittens.
5340/2 were 5 kittens in N environment, one of which went on to die of wet FCoVV
5340/3 were 5 kittens in M environment, 1 x 10, 3 x 20, 1 x 40, rising to 2 x 80, 2 x 160 at 16 weeks (no follow up for the kitten titre 10) born Mar 89, still fine 66 weeks after their first test at 12 weeks.

Table 5.4 Antibody titres of kittens which died of FCoVV

Ref.	Env.	Age	Titre	Age	Titre	Age	Titre	Age died	C/S	Wet/ dry
7001/1/1	M	8	160	8.5	20			8.5	C	Wet
5049/3/1	N	17	1280					17.1	C	Dry
5049/3/2	N	18	640	22	1280	26	1280	53.2	C	Dry
5145/2/1	N	13	10	20	80	30	160	30.5	C	Wet
5340/2/3	N	9	80	13	40	17	640	28.2	C	Wet
5343/2/2	N	10	20	14	10			13.5	S	
5476/2/2	N	9	0	15	80	26	640	32.0	S	
5686/1/1	N							13.8	S	
5692/1/1	N	1	80					1.8	S	
5762/2/1	N	11	1280					11.2	C	Wet
5762/2/4								9.8	S	Wet

Key:
Ref. = the kitten's reference number
Age = in weeks
Env. = environment
C = confirmed by histopathology
S = suspected of FCoVV on gross PM alone

Table 5.5 Fate of the kittens

		Alive			Dead		Unknown	
Env.	Sero pos/neg.	No.	No. weeks observed Mean in wks (range)		FIP	other	Sold	Unknown
N	+	80	53.5	(12.4-135.1)	5 (3xC)	4 (1 diarrhoea)	10	25
N	-	64	44.4	(16.2-110.7)	0	1 (RTA)	11	28
M	+	29	50.0	(6.1-71.1)	1 (C)	0	0	5
M	-	54	65.3	(13.8-147.2)	0	1 (CCF)	3	22
I	-	30	49.9	(14.5-87.8)	0	1 (RTA)	6	9

Key:
Env. = environment
+ = seropositive
- = seronegative
C = confirmed by histopathology
RTA = road traffic accident
CCF = congestive cardiac failure

was still well at 50 weeks; 1 was still seropositive 5 weeks after being sold and its antibody titre had fallen from 160 to 40; 5 were still seropositive at 22 weeks; 2 at 23 weeks; 1 at 24 weeks; 1 at 45 weeks. In one which was still seropositive at 25 weeks and showing neurological signs, FCoVV was suspected but PM revealed a porto-systemic shunt.

In the N environment, of the seronegative kittens which were retested after 16 weeks, 3 were still seronegative at 27, 33, 44 weeks of age. Six had become seropositive by 17 (this cat still seropositive at 21), 20, 33, 42, 42 (still seropositive at 57), and 45 weeks.
The fate of 5 seropositive kittens in the M environment was unknown. One was a littermate of the one which died and had an antibody titre of 10 at 10 weeks and zero at 13 weeks. Of the seropositive kittens which were retested after 16 weeks, 3 were still seropositive at 19 weeks; 1 at 21 weeks; 1 at 34 weeks; 2 of a litter of 3 were still seropositive at 17 weeks and the third was seronegative; 2 of 5 were still seropositive at 19 weeks, the other 3 were seronegative; one became seronegative by 30 weeks, remaining so at 48 weeks.

In the M environment, 5 of the seronegative kittens were still seronegative at 22; 23 and 27; 27; 37; and 39 weeks of age. Three kittens were sold 5.8 weeks after testing and no further data were available from them. Two became seropositive at 25 weeks; one was seropositive at 26 and 38 weeks and was still well at 70 weeks of age. One seronegative kitten (5144/4/1) died of congestive cardiac failure at a year old, its tissues showed no sign of FCoVV.

Of the 46 kittens in the I environment, one was killed on the road 32.4 weeks after testing; 4 were sold 2.7, 2.7, 9.5 and 10.1 weeks after testing and 2 were sold at an unknown time. Among those tested after 16 weeks, 3 were still seronegative at 25; 1 at 31 and 1 at 60 weeks of age.

Details of the health of the kittens, where available, is presented in table 5.6. Forty-one kittens failed to put on weight as normal, 33 of these were seropositive, 8 seronegative. Thirty-two had diarrhoea and 8 had not. Of the seropositive kittens, 28 had diarrhoea, 4 did not, and the status of 1 was unknown. Fifty-seven percent of kittens with diarrhoea were seropositive compared with 32% of kittens which had no diarrhoea. Statistical analysis revealed a causal relationship between diarrhoea and the presence of FCoV antibodies. This is significant by Chi-squared analysis ($p<0.5$).

Twenty-one of 26 kittens with URT signs or sneezing were seropositive and 2 went on to die of FCoVV. Calicivirus was isolated from 3 seronegative kittens. Calicivirus arthritis occurred in 3 seropositive kittens.

5.3.5 Kitten Mortality Complex

The prevalence of the main signs which make up the KMC are shown in Fig. 5.6. There is no difference between the proportion of seropositive queens which failed to conceive, aborted or resorbed, had stillborn or fading kittens and those which had none of these problems.

Eleven breeders reported failure to conceive in 13 queens. Four cats failed to conceive on more than one occasion. One of these was FeLV positive. Two cats were both seropositive and seronegative at different times of failure to conceive.

Abortion or resorption occurred in 12 cats belonging to 11 breeders and 2 cats were affected in more than one litter. Eighteen breeders reported stillborn kittens from 24 queens. Thirty-one queens had fading kittens according to their 21 breeders. Thirty-five breeders reported 89 litters which had none of these problems.

In table 5.7 the antibody status of littermates of premature,

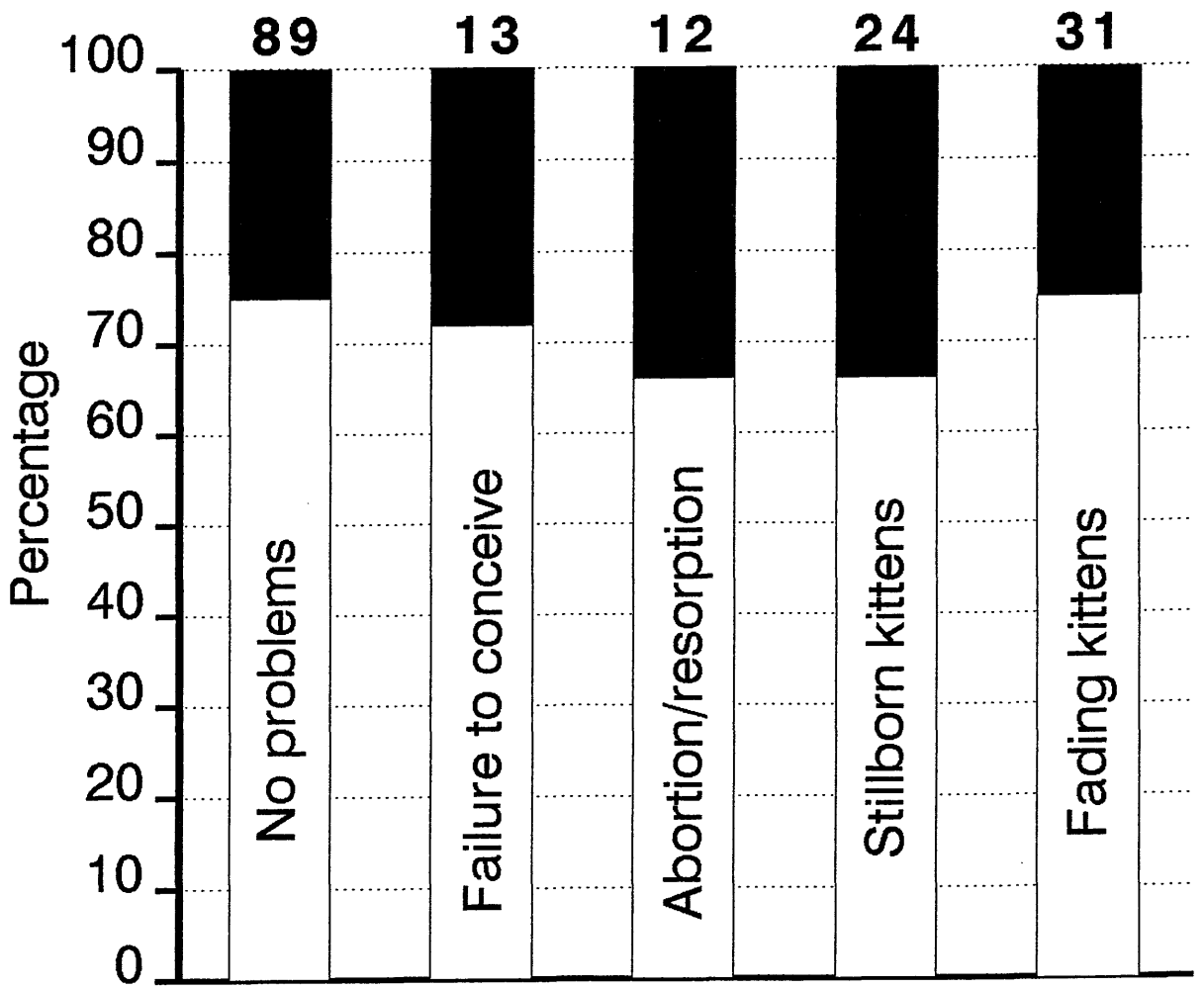
Table 5.6 Health status of the kittens

Condition	Seropositive	No. FCoVV	Seronegative	No. FCoVV
Completely healthy	35 (21%)	0	134 (79%)	0
No diarrhoea	73 (32%)	4	155 (68%)	0
Diarrhoea	59 (57%)	2	44 (43%)	0
Ocular discharge	4 (40%)	0	6 (60%)	0
URT signs/sneezing	21 (81%)	2	5 (19%)	0
Pneumonia			3	
Ataxia	2		1	
Ascites	2	2		

		No. diar.	No. FCoVV.		No. diar.	No. FCoVV
Poor growth	33 (80%)	28	1	8 (20%)	4	0
Normal growth	83 (32%)	26	0	180 (68%)	30	0

Key:
No. = number
diar. = diarrhoea
FCoVV = feline coronaviral-associated vasculitis
() = the percentage of the total with the given condition

Fig. 5.6



The incidence of various signs of the kitten mortality complex in survey cats

Key:

Seropositive

Seronegative

Table 5.7 Incidence of seroconversion of littermates in KMC

Ref no.		Queen's breed	Queen's titre	Littermates seropos/neg
Premature				
5692/2/2 5692/2/3 5692/2/4 5692/2/5	5 days premature 5 days premature 5 days premature 5 days premature	Persian	40	-
Stillborn				
5043/1/4		Foreign	160	+
5184/4/1		Burmese	0	-
5199/1/2		Siamese	0	-
5447/4/2*		Abyssinian	80	+
5469/4/2 5469/4/3 5469/4/4 5469/4/5		Russian Blue	160	-
5476/2/3		Abyssinian	0	-
5595/2/2		Persian	160	-
5626/3/1		Birman	80	-
5682/1/2 5682/1/3 5682/1/4		Persian	320	1 other in litter titre 10 at 6 wks
5703/1/3		Persian	80	+
5718/1/6 5718/1/7		Siamese	160	+
Fading kittens				
5045/2/5 5045/2/6		Burmese	320	+

Key:

The queen's reference number consists of the survey number of the queen, the number of the litters she has had to date and the number of the kitten within that litter.

\+ = seropositive
\- = seronegative

Table 5.7 contd.

Ref no.		Queen's breed	Queen's titre	Littermates seropos/neg
5127/1/2 5127/1/3 5127/1/4	11 wks imperf. anus 23d 23d	Chinchilla	0	-
5062/1/1	erythemic myelosis	British Blue	20	+
5198/1/2		Siamese	20	1 other - at 5wks
5351/1/3 5351/1/4 5351/1/5		Foreign	80	-
5444/9/1	pneumonia	Abyssinian	1	-
5447/4/3*		Abyssinian	80	+
5591/4/4		Persian	80	- (6wks)
5593/2/4 5593/2/5	1 day old cleft palate 1 day old	Persian	320	- (6 wks)
5596/3/2		Persian	20	-
5692/1/1	13 days, ? FCoVV	Oriental	320	+ at 2wks - at 12
5696/1/1	12.5 wks	Persian	0	+
5698/2/5		Persian	1280	+
5740/1/3 5740/1/4		Persian	320	+

Key:

The queen's reference number consists of the survey number of the queen, the number of the litters she has had to date and the number of the kitten within that litter.

\+ = seropositive
\- = seronegative

deformed, stillborn and faded kittens: antibody titre and breed of queen are presented. Histopathology was available for only a few of these kittens. Nine out of 23 litters seroconverted, showing presence of FCoV in the environment. Litter 5682/1 had one survivor which had titre 10 at 6 weeks, which is probably a tail-off of MDA (the queen had a titre of 320).

We received one aborted, 5 stillborn and 31 faded kittens from 17 households for histopathology and the results are presented in table 5.8. Histopathological evidence of FCoVV was found in only 1 of 31 faded kittens.

Table 5.8 Histopathology of kittens from seropositive households

Ref.	Age	Condition		Breed
1801		aborted	ND*	Persian
2863		stillborn	ND	Persian
1788		stillborn	ND	Persian
1686		stillborn	Spina bifida, malformation of vertebrae, myelodysplasia	Ragdoll
1785		stillborn	ND	Russian Blue
1679		stillborn	ND but thymus poorly developed	Burmese
1681		proliferative pneumonia		Siamese
1615	12h	ND		Persian
1782	4 w	FIE		Birman
1777		ND non-specific enteropathy and leukocytosis erythemic myelosis		
1686	14d	thymic atrophy, depletion of lymphoid organs		Ragdoll
		1 d lordosis		Ragdoll
1676		ND		Persian
1695	6 w	bacterial peritonitis		Somali
	4 w	acute exudative bronchopneumonia		
		acute exudative bronchopneumonia		
		acute exudative bronchopneumonia		
1790	3 d	ND		Birman
1596	1 d	ND		Devon Rex
1594		ND		Colourpoint
		ND		Colourpoint
Hart	2 w	FCoVV		Devon Rex
Hughes	4 w	ND		Siamese

Key:

***ND** No evidence of any infectious disease including FCoVV.
d = days
h = hours
w = weeks

Table 5.8 contd.

Ref.	Age	Condition	Breed
1598	4 d	ND	Siamese
	4 d	ND	Siamese
1690	1 d	ND	Persian
	1 d	ND	Persian
	1 d	ND	Persian
	1 d	ND	Persian
1774	5 w	thymic atrophy, calicivirus inf'n	Abyssinian
	5 d	smothering	Abyssinian
	1 d	lordosis	Abyssinian
	5 d	ND	Abyssinian
	2 w	exudative bronchopneumonia	Abyssinian
	3 w	exudative bronchopneumonia	Abyssinian
		pneumonia	Abyssinian
	2 w	exudative bronchopneumonia	Abyssinian
	2 w	exudative bronchopneumonia	Abyssinian

Key:

***ND** No evidence of any infectious disease including FCoVV.
d = days
h = hours
w = weeks

DISCUSSION

5.4.1 Waning of MDA and seroconversion of kittens

In those kittens which were seronegative at 8,9, and 10 weeks and seropositive a few weeks later, it was impossible to ascertain whether this reflected later infection or delayed seroconversion.

In litter 5340/2, 5 kittens were seropositive at 9 weeks and 3 became seronegative by 13 weeks. One of the two which remained seropositive went on to die of FCoVV. Since the queen's antibody titre around the time of birth of these kittens was 40, it is unlikely that the kittens' antibody was maternally derived. ($T1_{/2}$ of MDA in FCoV infection is 7 days [111].) It would appear, therefore, that all of the litter were exposed to later FCoV infection and seroconverted, but most of them recovered and soon became seronegative. A previous report of FCoV infection in kittens noted that IFA titres peaked rapidly then dropped to undetectable levels in a month or so while virus neutralising antibody titres remained high [112].

The serological pattern of litter 5340/2 is consistent with experimental findings in which sublethal doses of FIPV were given to cats so that some animals recovered, while others succumbed. Mortality did not increase with increasing dose in a predictable way, suggesting that host susceptibility is as important as virus dose in the development of the disease. [112].

Queen 5340 went on herself to die of FCoVV. In previous surveys it was noted that when several cats from the same colony were affected they tended to either be littermates or successive litters from the same queen [101, 116, 140]. One explanation for this could be that there may be genetic predisposition to the disease.

The kittens in litter 5476/2 were seronegative at 9 weeks

though 5476/2/2 was seropositive at 15 weeks and went on to die of FCoVV. In the same household only 2 of 6 in litter 5473/1 were seropositive at 12 weeks. This finding is similar to the experimental situation in which FIPV was shown to have low infectivity but high mortality [110]. Later experiments showed this effect to be dose related [112]. Interestingly, these two litters were kept in a normal environment. If the kittens were infected by an adult other than their mother, it might be expected that exposure would be more sporadic and the virus dose smaller.

To obtain a more accurate assessment of their infection status, kittens should be tested at 10 weeks of age or over and where possible they should be tested twice at a four week interval.

5.4.2 Influence of environment

In the literature, reports of naturally occurring FCoV infection in kittens are rare and the route of transmission has remained speculative [97, 98, 110].

As in our preliminary report of 200 kittens [1], in this survey, the majority of seropositive kittens and most of those which died of FCoVV were from households which had allowed the kittens to mix with adult cats other than their mothers. Those kittens which were isolated from all adults in the household were all seronegative. This suggests that transmission of coronavirus to kittens generally takes place horizontally after birth, in most cases from adults other than the queen. Four seronegative queens in the normal environment had kittens which became seropositive, supporting this theory.

As shown in table 5.2 this finding held true for successive litters from the same queen. The likelihood of seroconversion of the kittens altered predictably as the environments changed between successive litters, regardless of the antibody titre of the queen.

Our results indicate that in FCoV seropositive households the

queens and her kittens should be kept in isolation from all other cats until sold.

5.4.3 Virus excretion by queens

Since 11 litters from 30 seropositive queens (37%) in the M environment were seropositive, it would appear that approximately one in three seropositive queens excrete virus. The antibody titre of the queen was not an indicator or whether or not she was excreting virus. One queen which had two litters in the M environment became seropositive between the first and third litters and the kittens in these litters remained seronegative. However her second litter was reared in a N environment and seroconverted. Unfortunately, because the kittens of the second litter mixed with adults other than their mother, it is not known whether she went through a transitory phase of virus excretion after infection. Her antibody titre was 1280 at the time of the second litter and 320 at the time of the third.

Four of the virus-excreting queens (5015, 5419, 5587 and 5717) became seronegative 11.5 months after the test nearest the birth of the seropositive litter (range 10-13 months). This result indicates that virus excretion does not always last for life. Cat 5419 had been seropositive for 11 months before the litter was born, therefore had been seropositive a total of at least 26 months, though it is not known whether she was excreting virus continuously throughout that time.

Cat 5111 had been seropositive for 20 months before giving birth. Circumstantial evidence indicates that she might be a virus excretor during this time, so that the litter was planned in order to confirm these suspicions.

No seronegative queens appeared to excrete virus.

5.4.4 Health of the kittens

In this study it was shown that FCoV is associated with diarrhoea in kittens. Diarrhoea occurred in kittens from households which had no history of FCoVV or FECV as well as those which did. Experimentally, enteritis due to FIPV or FECV occurred 2-3 days after infection [48, 111] which would be well before a serologic response would be expected; therefore, the prevalence of FCoV infection in diarrhoeic kittens may be higher than was found here.

Failure to gain weight was linked both with diarrhoea and the occurrence of FCoV antibodies.

No particular clinical signs in kittens were noted more frequently in those kittens which went on to die of FCoVV.

It was impossible to know how many seropositive kittens which were sold were subject to reinfection in their new homes but the majority were still well almost a year later. It could not be determined whether their initial exposure rendered them more susceptible or resistant.

5.4.5 FIPV/FECV?

One reason for proposing the existence of FECV was the apparent discrepancy between the low incidence of FCoVV and the high incidence of seroconversion in the field [102]. In the laboratory, FIPV had been shown to have a low infectivity but a high mortality, whereas FECV had high infectivity and low mortality [111]. However, in these experiments, increasing the dose of FIPV increased infectivity and mortality [106, 112] and later isolates of FIPV had high infectivity and low virulence [114].

Because virus isolation in the field is almost impossible, it was unknown whether the seropositive households in the survey had FECV, FIPV or indeed either virus present at the time of study. However, 31 out of 41 of the households had a history

of a case of FCoVV, often at the beginning of the survey, so virulent FCoV must have been present at some time. The prevalence of infection in those litters which were infected was 92% in N environment and 90% in M environment. Thus, if two different viruses, FIPV and FECV, exist outside of the laboratory their patterns of infection are indistinguishable, so they presumably occur together.

When experimental cats were dosed with sublethal amounts of FIPV, they either did not become infected, seroconverted without developing the disease or developed FIP [112]. This paradigm closely resembles the sporadic and low incidence of disease in nature, suggesting that cats are exposed to relatively small amounts of virus [106].

Although FCoV was shown to be associated with diarrhoea, no difference in the incidence of FCoVV was evident between households where clinical history indicated FECV infection and those which indicated FIPV infection.

It could be concluded that FCoVV is a sporadic manifestation of a common, asymptomatic or enteritis-causing FCoV infection. If there are truly different FCoVs in the field of varying virulence, then they occur together so often that to postulate their presence in isolation causes unnecessary confusion to veterinary surgeons and cat owners, difficulty in interpreting serology and a false reason for complacency among some breeders of seropositive kittens.

5.4.6 Kitten Mortality Complex

While the incidence of seropositivity of queens which failed to conceive, had aborted/resorbed, or had stillborn, faded or deformed kittens seemed high (72%, 64%, 66%, 79% and 86% respectively) it was virtually the same as the incidence in those queens which had none of those problems (75%.) Therefore, FCoV is unlikely to be a cause of these conditions.

There were no cases of cardiomyopathy in the kittens from seropositive households examined by histopathology and only one had evidence of FCoVV.

Seventy-four percent of litters with premature, stillborn or fading kittens where antibody status of littermates was known had no evidence of FCoV infection.

In conclusion, as found in a previous investigation [114], it seems unlikely that FCoV is a cause of KMC.

CHAPTER 6

THE SEROLOGICAL RESPONSE OF CATS TO INDIVIDUAL STRUCTURAL PROTEINS OF FCoV

6.1 INTRODUCTION

As shown in Chapter 4, no difference in antibody titres by IF could be discerned between cats which succumbed to FCoVV and those which survived. It was decided to examine the nature of the antibody response in more detail, because it had been postulated that anti-S antibody might be enhancing antibody [149] and therefore it was possible that cats which survived FCoV infection would lack this antibody. Although it has been stated that animals which died of FCoVV and those which were considered to be immune had comparable humoral reactivity, the S protein was only detected when certain, undefined, serum samples were used [15]. Ishida noted that activity to a 95K protein was found on immunoblots in 14 of 23 FCoVV cases but only 3 of 39 healthy seropositive cats. The 95K protein may be a proteolytic product of S or monomeric form [67, 68].

Alternatively, surviving cats might possess an antibody which conferred protection and which those cats which succumbed may lack. A marker such as the presence or absence of a particular antibody would be a useful tool in the field for differentiating healthy, seropositive cats from those which are likely to succumb to FCoVV.

In this chapter, experiments to investigate the presence of antibodies to the individual FCoV proteins are described. This was done by immunoblotting in which the virus was disrupted in reducing buffer, its components were separated using PAGE and tranferred to a nitrocellulose membrane which was then probed with the serum samples to be tested.

Our results indicated that there may be a difference between the level of anti-M antibody in cats which succumb to FCoVV compared to cats which survive the infection. Those which succumbed appeared to have more anti-M antibody. However, quantification of antibody was difficult using immunoblotting and it was concluded that further work was needed to gain accurate antibody titres to each of the viral components.

6.2 MATERIALS AND METHODS

FCoV was concentrated as described in 2.10 and quantified by comparison with known concentrations of ovalbumin on PAGE as described in 2.8 and illustrated in fig 2.1. Immunoblots were made and developed as described in 2.10 and 2.11.

The MAbs 52D5 which recognises the FCoV N protein, 6F7.1 against M and 9A1.1 which is anti-S were kindly donated by Dr Mike Bartkowski, Fermenta Animal Health, Omaha, NE 68134. The ascitic fluid sample from a histopathologically confirmed case of FCoVV was kindly supplied by Miss Ann Hutchison. This sample was designated 'Mostafa' after the owner of the cat.

Sera were from the survey described in Chapter 4 and from routine samples submitted to the FVU for FCoV IF and histopathology. Thirty serum samples from 6 cats involved in a temperature sensitive FCoV vaccine experiment were kindly supplied by Dr Jay Gerber, Smith Kline Beecham, Lincoln, Nebraska NE 68501.

6.3 RESULTS

6.3.1 Viral proteins

The authenticity of FCoV proteins on immunoblot were confirmed by using MAbs and the results are shown in fig 6.1. From this it is seen that the S forms a band at approximately 210K, N at 46K and M forms a diffuse band between 24-30K. Immediately above the S band, another, fainter band can be seen on track 1 which is probably an aggregate of the M protein which has failed to enter the gel [144]. A similar band appears more plainly in fig. 6.2 where the anti-M MAb 6F7.1 identifies it as M.

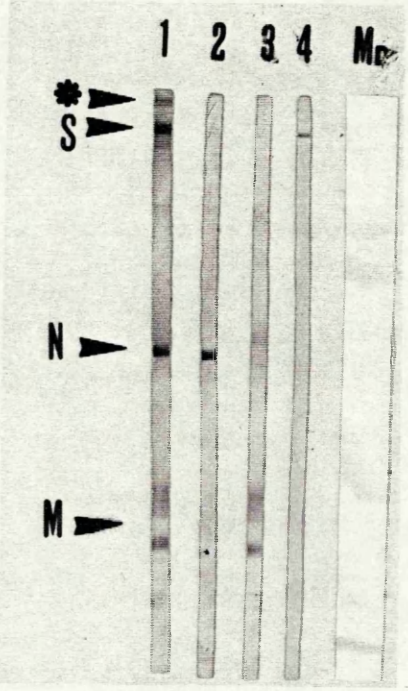
6.3.2 Immunoblots of survivors and cats with FCoVV

In the first experiment the reactivity of 6 sera from cats which had died of FCoVV were compared with that of 8 samples from 7 in-contact survivors. These results are presented in table 6.1. The samples from 3 survivors (5027, 5605, 5691) and 2 dead cats (Edwards, Canham) reacted with bands at 210K and these 5 cats and 2 more cats also produced bands at 95K. All of the samples except one (Carroll 2) had anti-N antibody. Of interest, however, was that while only one survivor (5364) had a strong M band, 4 of the FCoVV cases had strong anti-M. In three survivors (5027, 5542 and 5691) a weak M band could be detected.

Four samples from each of these groups were tested again (Experiment 2). In this case, all 4 of the dead cats had anti-S, anti-N and anti-M antibodies. Only one of the survivors (5364) had a strong M band as well as N, though in two (5027, 5357) a faint M could be detected. Two survivors (5364, 5605) had strong and one (5357) a faint anti-S band.

Four sera from FCoVV cases were reacted in a third experiment in which 3 (Edwards, Carroll 1 and Matsell) had a M band, all 4 had N and 2 (Edwards and Carroll 1) had S bands. Thirteen more samples from 9 cats which had died of FCoVV were then blotted.

Fig. 6.1



The S, N and M proteins of FCoV. Above the S is a fainter band (marked *) which is M aggregate which has failed to enter the running gel.

1. Mostafa ascites
2. MAb 52 D5 (anti-N)
3. MAb 6F 7.1 (anti-M)
4. MAb 9A1.1 (anti-S)

Mr Molecular weight markers

Table 6.1 Immunoblots on sera from cats which developed or survived FCoV infection.

Sample ref	Cat	Household	IFA titre	M 1	M 2	M 3	N 1	N 2	N 3	S 1	S 2	S 3	95K 1
Cats which died of histopathologically confirmed FCoVV													
P1409C	Edwards		1280	++	++	++	++	++	++	++	++	++	++
P1112C	Carroll 1		640	++	++	++	++	++	+	-	++	++	-
P3587C	Matsell		1280	+	++	++	++	++	++	-	++	-	++
P1915C	Canham		1280	-	++	-	++	++	++	+	++	-	++
P3301C	Horton	3123	1280	++			++			-			-
P1154C	Hancock	3122	320	++			++			-			-
P6670B	5080	1615	1280			++			++			-	
P2329C	5097	1615	1280			++			++			++	
P6995B	5376	1685	1280			++			++			-	
P9140B	5376	1685	1280			++			++			+	
P8276B	5466	1776	20			++			+			-	
P7475B	5481	1777	320			-			++			-	
P348C	5481	1777	1280			++			++			++	
P2126C	5481	1777	1280			++			++			++	
P8505B	5340/2/3	1768	640			+			++			-	
P9930B	5340/2/3	1768	640			++			++			-	
P1743C	McTavish		320			++			++			-	
P1977C	5716	3121	1280			++			++			-	
P4822C	Hancock	3122	320			++			++			+	
In-contact surviving cats													
P6996B	5357	1685	640	-	+		++	++		-	+		-
P7026B	5027	1596	1280	+	+		++	++		+	-		++
P7495B	5605	1789	320	-	-		++	++		++	++		++
P2927C	5605	1789	640	-		+	++		++	+		-	
P9127B	5364	1685	1280	++	++		++	++		-	++		-
P6985C	5542	1685	1280	+			++			-			-
P1617C	569	3095	1280	+			++			+			++
P1058C	Carroll 2		640	-		-	-		-	-		-	+

This table shows that anti-M antibody was usual in cats which succumbed to FCoVV and less common in cats which survived. It also shows that while anti-N activity was reproducible from immunoblot to immunoblot, anti-S and anti-M were more variable. A band at 95K was only visible on blot 1.

Key:
M = integral membrane glycoprotein N = nucleocapsid S = spike
1, 2 and 3 = Experiments 1,2 and 3.
++ = strong band
+ = faint band
- = no band

All but two had strong M bands and all had N bands. Three also had good S bands and two had faint S bands.

Of particular interest in the third experiment is cat 5466, which had a IFA titre of 20 in July 1988 and died of FCoVV in November 1988. This sample had a strong M band and a weak N band.

In order to increase the numbers of survivors for comparison, sera from cats in the survey household 1789 and cat 5600 from household 1788 were then immunoblotted (fig 6.2). On this blot, a dramatic contrast is evident between cats which died of FCoVV in Feb 1989 (5611 and 5660) and the other cats which were still alive in Mar 1991. Cat 5611 which developed FCoVV had noticeable anti-M antibody even 12m before developing FCoVV whereas the survivors had very little evidence of anti-M activity even in sera with fairly high IFA titres. No anti-S was visible although the molecule had transferred to the blot because it was detected by the MAb 9A1.1. Bands were visible at 95K on several samples which may or may not have been monomeric S or a reduced product of S, although the anti-S MAb did not recognise it.

After conflicting results of immunoblots from other households, the sera from household 1789 were retested and the two blots of this retest are shown in fig 6.3. Anti-M activity is evident in most samples though it is still not present in cats 5605, 5609 and 5784 and is faint in others. In cat 5600 anti-M was seen to diminish as the cat's IFA titre fell from 640 to 40. Cats 5611 and 5660 show anti-S activity as do 4 of the 5 confirmed FCoVV sera and 9 of the 16 survivors. In sera of two of the survivors the S band is very faint indeed.

Also included in fig.6.3 are 5 non-survey confirmed cases of FCoVV. Interestingly, the last of these samples was from an IFA negative cat in which only anti-N activity is evident.

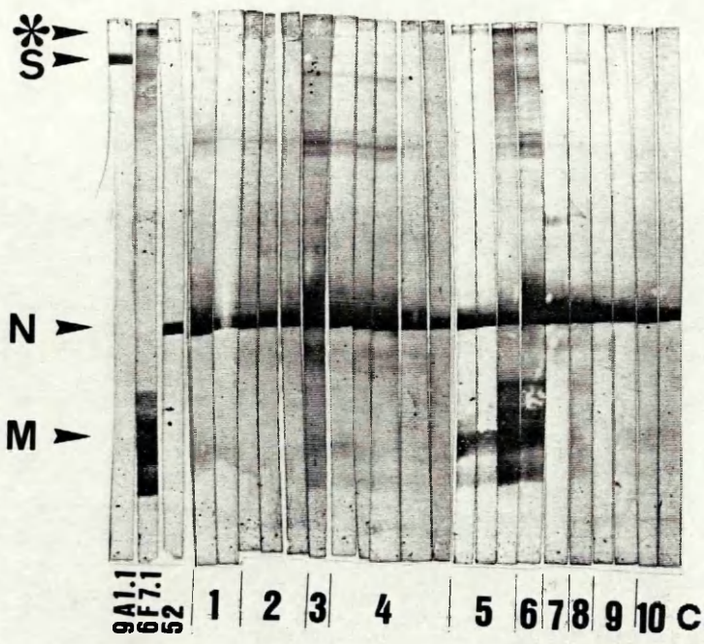


Fig. 6.2

Immunoblot of sera from 7 cats from households 1788 and 1789 which survived and 2 which succumbed to FCoV infection. This blot shows less anti-M activity in surviving cats than in those which died.

Antibody titres and clinical history of cats 1-10

1. 5614
   Jun 88 640
   Feb 89 320
   Well Sep 90

2. 5615
   Mar 88 160
   Jun 88 80
   Mar 89 40
   Well Sep 90

3. 5619
   Feb 89 320
   Well Sep 90

4. 5600
   Apr 88 1280
   May 88 1280
   Jun 88 640
   Aug 88 320
   May 89 40
   Well Sep 90

5. 5611
   Mar 88 320
   Jun 88 320
   Feb 89 1280
   Died Mar 89 FCoVV

6. 5660
   Feb 89 1280
   Died Mar 89 FCoVV

7. 5605
   Feb 89 640
   Well Apr 90

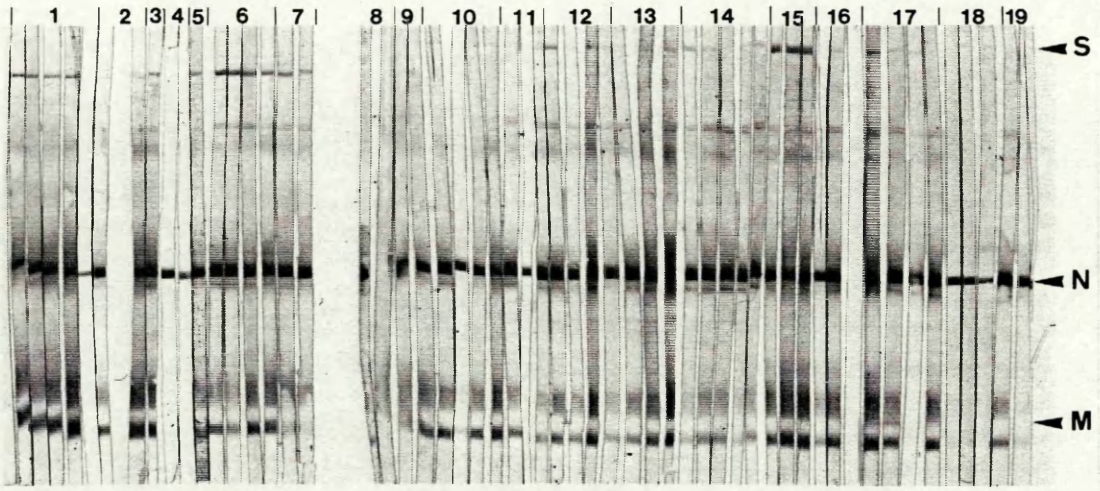
8. 5606
   Jun 88 640
   Died kidney failure Apr 90

9. 5607
   Jun 88 320
   Feb 89 640
   Well Sep 90

10. 5608
    Jun 88 160
    Mar 89 20
    Well Sep 90

* is M aggregate which has failed to enter the running gel.

Fig. 6.3



Repeat immunoblots of sera from cats in household 1789. This blot shows anti-M activity in both surviving cats and those which died.

IFA antibody titres and clinical histories of cats 1-19

1. Non- survey cats with FCoVV
   Ranger 1280
   Symonds 4 1280
   Gledhill 320
   Buchanan 1280
   P888D 0

2. 5611
   Jun 88 320
   Feb 89 1280
   Died Mar 89 FCoVV

3. 5660
   Feb 89 1280
   Died Mar 89 FCoVV

4. 5605
   Jun 89 40
   Sep 89 160
   Well Apr 90

5. 5606
   Jun 88 640
   Died kidney failure Apr 90

6. 5607
   Jun 88 320
   Jun 89 320
   Sep 89 640
   Mar 90 320
   Well Sep 90

7. 5608
   Jun 88 160
   Mar 89 20
   Well Sep 90

8. 5609
   Mar 88 40
   Mar 90 10
   Well Sep 90

9. 5610
   Jun 88 80
   Mar 89 20
   Well Sep 90

10. 5613
   Mar 88 160
   Jun 88 320
   Feb 89 320
   Sep 89 640
   Well Sep 90

11. 5614
   Jun 88 640
   Feb 89 320
   Jun 89 80
   Well Sep 90

12. 5615
   Mar 88 160
   Mar 89 40
   Sep 89 640
   Mar 90 20
   Well Sep 90

13. 5619
   Feb 89 320
   Sep 89 160
   Mar 90 640
   Well Sep 90

14. 5602
   Jun 88 80
   Mar 89 160
   Jun 89 80
   Sep 89 80
   Mar 90 40
   Well Sep 90

15. 5664
   Feb 89 80
   Sep 89 80
   Mar 90 80
   Well Sep 90

16. 5616
   Jun 88 20
   Mar 89 80
   Jun 89 20
   Well Sep 90

17. 5612
   Jun 88 80
   Mar 89 0
   Sep 89 20
   Mar 90 20
   Well Sep 90

18. 5784
   Mar 89 80
   Sep 89 20
   Mar 90 0
   Well Sep 90

19. 5600
   Jun 88 640
   May 89 40
   Well Sep 90

Thirty serum samples from 6 cats involved in a temperature sensitive FCoV vaccine experiment were also blotted twice. On the first blot (fig 6.4) anti-S and anti-M activity were only apparent in the sera of cats 2 and 4 which were the only cats to develop FCoVV. On the second blot (fig 6.5) anti-S activity was evident in all cats except the unvaccinated, unchallenged control (no. 5) and a cat which was not vaccinated and did not develop FCoVV post challenge (no. 3). Anti-M was evident in all of the cats which were challenged and faintly and briefly in cat 6 which was vaccinated but not challenged.

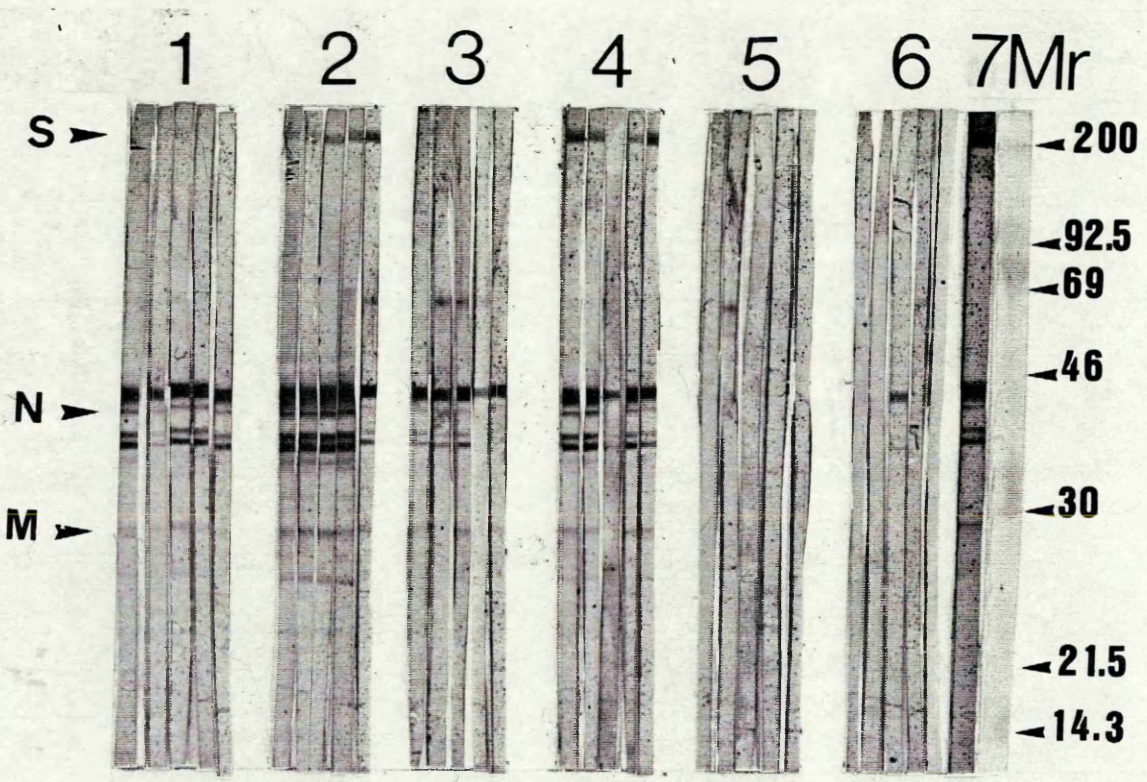
At this stage it was necessary to assess whether or not the disparity in band appearance was related to the quantity of viral protein on an individual immunoblotting membrane. Virus was titrated in doubling dilutions against two sera (P7926B and P2923C) which had given disparate results. The results are shown in fig.6.6. Clearly these sera cannot detect low quantities of S and M, although they can still detect N. There are several possible explanations for this result: there may be a greater amount of N protein in the initial purified FCoV sample; N may be more readily transferred from gel to nitrocellulose; the immunogenic epitopes of N may be conserved more during PAGE than the epitopes of M or S or N may be more immunogenic than M or S; or titres of anti-M and anti-S antibodies may be much lower than anti-N titres.

After this experiment the amount of viral protein used on each gel was increased to 35ug per gel so that low levels of anti-S and anti-M antibody could be detected. However, the gels were still difficult to standardise due to inconsistency of M entering the gel.

6.3.3 More immunoblots of survivors and cats with FCoVV

Using this revised protocol a further 26 samples from 6 cats which died of FCoVV and 262 samples from 75 surviving cats from 21 households were immunoblotted. Of the cats which died, all 26 had anti-N and in addition 22 samples had anti-M. Two

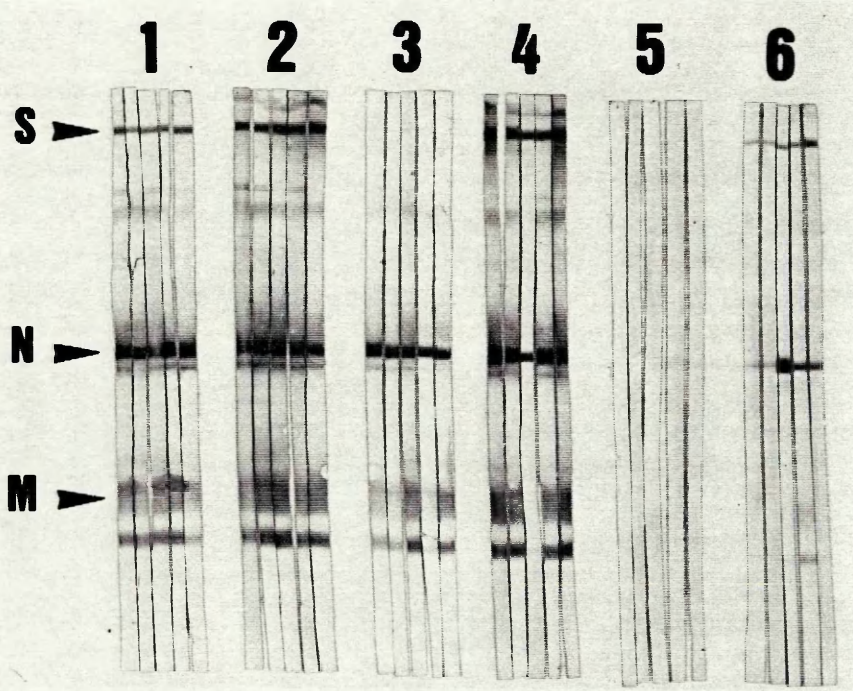
Fig. 6.4



Immunoblot of sera from a temperature-sensitive FCoV vaccine experiment showing more anti-M activity in cats 2 and 4 which developed FCoVV than in those which survived.

1. vaccinated, did not develop FCoVV post challenge
2. vaccinated, developed FCoVV post challenge
3. not vaccinated, did not develop FCoVV post challenge
4. not vaccinated, developed FCoVV post challenge
5. not vaccinated, not challenged
6. vaccinated, not challenged

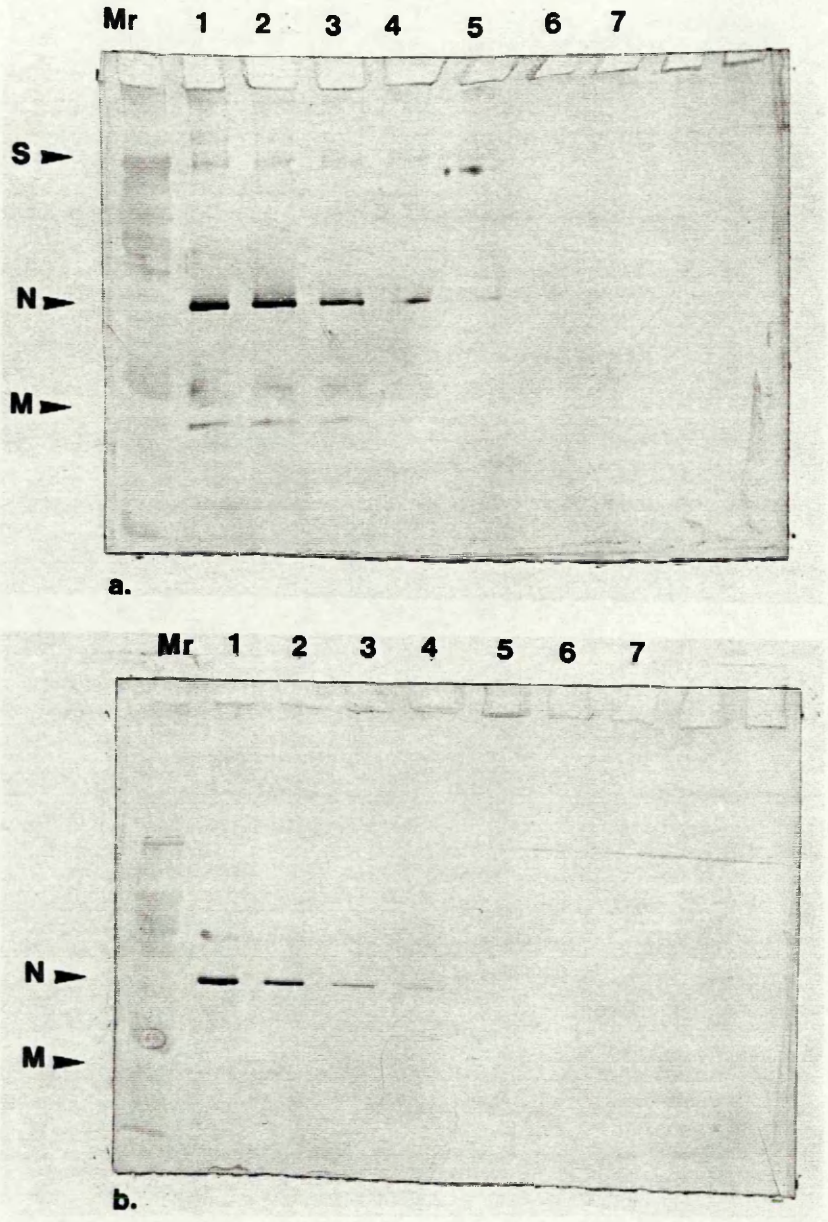
Fig. 6.5



The second immunoblot of sera from a temperature-sensitive FCoV vaccine experiment showing anti-M and anti-S activity in samples in which it was not apparent in fig. 6.4.

1. vaccinated, did not develop FCoVV post challenge
2. vaccinated, developed FCoVV post challenge
3. not vaccinated, did not develop FCoVV post challenge
4. not vaccinated, developed FCoVV post challenge
5. not vaccinated, not challenged
6. vaccinated, not challenged

Fig. 6.6



FCoV dilutions developed against P2923C (a.) and P7926B (b.) showing the disappearance of anti-S and anti-M activity at lower concentrations of FCoV on the blot.

Mr Molecular weight markers

1. FCoV undiluted
2. FCoV 1:2
3. FCoV 1:4
4. FCoV 1:8
5. FCoV 1:16
6. FCoV 1:32
7. FCoV 1:64

hundred and fifty-eight of 262 survivor samples had anti-N and 213 had anti-M antibodies. A complication was the variable accumulation of M at the top of the gel, very close to the S band and easily mistaken for S.

Unfortunately, the anti-M MAb did not detect M on some blots. Although the Mostafa ascites did, it was sometimes difficult to be certain whether higher MW bands contained S or M. However, of the blots where a S band was clearly identifiable, 8 of 26 FCoVV samples and 92 of 219 survivors had anti-S antibodies.

A band at 95K appeared on 90 strips cut from blots which had been cut into a total of 195 strips. Only 22 of these appeared to lack p220. Seventeen of these 22 strips were on blots on which the S band was very faint and very rare. The postulate that p95 is a proteolytic product of S is therefore quite feasible. Fifteen of 26 samples from cats which died of FCoVV and 75 of 169 survivors had anti-p95 antibodies. In a few blots a band was also visible at 69K, which may have been another fragment of S or could have been a product of the cell culture in which the virus was grown.

Of interest in these experiments were sequential samples from cats. Cat 5361 had slowly falling IFA titres and anti-N antibody only. Appearance of anti-S and anti-M for the first time in 3 years of sampling coincided with a rise in IFA titre from 80 to 640 suggesting that a new infection resulted in the appearance of anti-S and anti-M antibodies. In fig. 6.3, loss of anti-S antibody can be seen in cats 5612 and 5602. In cat 5615 anti-S antibody can be seen to disappear as the IFA titre falls and rises again. Anti-M can be seen to increase in cat 5602 as anti-M falls although the IFA titres remain about the same. Anti-M disappeared in cat 5600 as the IFA titre fell from 640 to 40.

6.3.4 Samples from cats of known virus excreting status

It was noted that sequential samples from cats often showed loss of anti-M and anti-S antibodies as these cats became IFA negative. It therefore seemed possible that cats with only anti-N antibodies were not excreting virus. To test this hypothesis samples from queens which had had kittens isolated with them and therefore whose virus excreting status was known, were examined.

Nine samples from 7 queens which had seropositive kittens and 13 samples from 10 queens which had seronegative kittens were immunoblotted. (Where more than one sample was available around the time of the kittening, all samples were used.) Anti-M was present in 9/9 samples from queens with seropositive kittens and 10/13 samples from queens with seronegative kittens. Anti-S was present in 3 queens with seropositive kittens and 2 with seronegative kittens. Anti-N was present in all samples except one from a mother of seronegative kittens.

Cats in household 3125 which became IFA negative, and therefore which had not been excreting virus, had anti-M antibody on many tests. Anti-S antibody was only apparent on 2 strips.

It was concluded that whether or not a cat was excreting virus could not be deduced when antibodies to 2 or 3 of the viral proteins were present.

6.3.5 Presence of antibodies in IFA negative cats

Of 35 samples from IFA negative cats, 19 had anti-M, 33 had anti-N and 2 had clear S bands and 4 had faint S bands. Samples from two IFA negative confirmed cases of FCoVV (cats 5040 and 5508) had only anti-N antibody. It seemed appropriate then to examine a household which was predominantly IFA negative.

All of the cats of houshold 1679 were immunoblotted. In this household all the cats eventually became IFA negative and all kittens that were born into the household were IFA negative

therefore it was concluded that these cats were not excreting virus. Cats 5186 and 5187 which remained IFA positive longer than the others were kept in isolation until they too had no antibodies detectable by IF. Two kittens and 2 new cats introduced to the household were included in the immunoblot.

Only the samples from the introduced cats and one sample from 5184 showed no reactivity. The other samples had anti-N antibody and sera from cats 5182, 5186, 5187 had anti-M activity. Cat 5184 had anti-M but subsequently lost it. Cats 5186 and 5187 had anti-S antibody. Cat 5183 never showed IFA activity and cats 5184, 5185 and 5188 were only IFA positive each on one or 2 occasions but consistently had anti-N antibody. Anti-N antibodies can plainly persist for longer than 2 years 3 months. Seven samples from 4 IFA negative cats had anti-M antibodies, probably reflecting the greater sensitivity of immunoblotting compared to IF. It appeared that cats with only antibodies to the N protein were no longer excreting virus.

6.4 DISCUSSION

6.4.1 Viral proteins on immunoblots

Considerable difficulty was encountered in standardising the FCoV immunoblot results: two gels from the same virus preparation run down each side of the Miniblot cell at the same time and blotted in exactly the same way did not appear to have the same intensity of virus bands, particularly the M and S proteins. Quantities of M often failed to travel past the end of the stacking gel.

Previously Sturman reported this phenomenon for p23 (M) of the murine coronavirus A59 after boiling virus samples in the presence of high levels of reducing agents [144]. However, in the present study, an experiment with and without 2-ME in the sample buffer and boiling for 1,2 and 3 mins or incubating at 37^{o}C for 15, 30, 45 or 60 mins failed to reveal any marked improvement in the quantity of M entering the gel for any particular method. Hence boiling for 3 mins in presence of 2-ME was continued.

Other authors have reported difficulty measuring the anti-S response of cats and various explanations have been offered: loss of critical antigenic determinants during SDS-PAGE or electrophoretic transfer [15, 59]; preferential induction of low-affinity antibodies by S [59]; or lack of humoral response to S [15]. The first of these explanations could not be applied to most of the immunoblots used here because some sera did recognise S. Different serotypes with different S epitopes are also unlikely to explain the variation in activity against S since sera from cats within the same household were usually compared on the same immunoblot and one would not expect great variation in serotype within a single household. Therefore, it would appear that many cats either mount no antibody response to S or produce only low-affinity antibodies.

6.4.2 Immunoblots of survivors and cats with FCoVV

Despite this problem, comparison of antibody activity of various samples was still possible within the same immunoblot and showed that most cats which developed FCoVV had higher levels of anti-M and anti-S activity than in-contact cats which survived because their sera picked up lower amounts of virus on blots than did the sera of survivors.

Higher levels of anti-M and anti-S activity could reflect active viral replication at the time of sampling. Alternatively, these antibodies could be responsible for enhancing disease. However Vennema et al did not find that recombinant vaccinia expressing M caused ADE on challenge [149].

When the quantity of virus on blots was increased, no difference in antibody response could be discerned between cats which survived or succumbed to FCoV infection. Anti-S and anti-M appeared in samples from cats in each group.

Unfortunately, antibody titres to various virus proteins cannot easily be obtained using immunoblotting and further work is required to establish whether the difference in anti-M and anti-S antibodies between surviving and succumbed cats would be useful to establish prognosis. However, the pathogenesis of this disease must be borne in mind since after damage to blood vessels occurs and before death has resulted from that damage, virus may be eliminated from the body and IFA titres may return to zero.

Antibodies to p220 and p95 were present in many survivors as well as cats which died. It seemed likely that p95 is a breakdown product or possibly a monomeric form of p220 since most antibodies which recognised the former also recognised the latter. Those strips which showed p95 and not p220 were from blots where no antibody illuminated p220 well, suggesting that most of the p220 had been broken down to p95 during processing.

The possibility that the S of FCoV is a dimer has been mentioned by two authors [15, 67].

6.4.3 Presence of antibody in IFA negative cats

It was noted that anti-N activity remained long after cats became IFA negative. Many authors have reported that IF activity is due to anti-M antibodies [59, 163] and this is one possible explanation for the absence of IF activity in cats with no anti-M antibody. Alternatively, it could be that immunoblotting is more sensitive and detects lower levels of antibody than IF. Since estimates of FCoV prevalence have been based on IFA readings, it is probable that the real prevalence in infected households is even higher than previously believed.

In this study it was shown that cats with only anti-N antibodies were not infectious to other cats. However, the converse was not true: cats with antibodies to S and M were not necessarily infectious. Unfortunately, to apply this information to cats in the field one would have to be sure that each blot had sufficient S and M protein to reveal the presence of even small amounts of antibodies against S and M.

6.4.4 Conclusion

In this study it was shown that there is variation in the antibody levels in different cats to each of the viral proteins of FCoV. Many cats which succumb to FCoVV seem to have higher levels of anti-M antibody than do cats which survive and this could be apparent as much as twelve months before the animal died. Higher levels of anti-M may represent increased systemic, as opposed to gut, viral replication. Alternatively, it is possible that the production of anti-M antibody in these cats has an enhancing effect which promotes increased infectivity.

However, there were cats which lost all anti-FCoV antibodies except anti-N and still went on to die of the lesions sustained during the initial infection. Therefore it has to be

remembered that the nature of antibody response is not prognostic.

Cats which only had detectable levels of anti-N, in the absence of antibodies to the other FCoV proteins, appeared not to be infectious. This finding could be useful in situations where a cat owner has lost one cat and wishes to know if a remaining cat is infectious in order to purchase a replacement or where a cat breeder wishes to know if it is safe to breed from a seropositive animal.

Further work is required to elucidate these findings. We propose to devise an IF test based on recombinant vaccinia-expressing each of the three FCoV proteins so that accurate titres can be found.

CHAPTER 7

GENERAL DISCUSSION

The primary aims of this research were to evaluate the usefulness of the IFA test in the field and to determine the fate of cats with antibodies to FCoV.

Surprisingly, for a disease which has been recognised for thirty years, this study was the first longitudinal survey of naturally infected cats in the field. The results obtained answer many of the questions which trouble practicing veterinarians and revealed simple management techniques which could reduce the incidence of infection and disease. It was also found that the situation in natural infections was quite different, in a number of areas, to the picture which had previously been obtained by extrapolating from experimental situations.

At the beginning of the research, the usefulness of the IFA test had been called into question chiefly because of the assertion that many strains of FCoV existed: a virulent form called FIPV, one which caused diarrhoea called FECV and yet others which were associated with asymptomatic infections. The clinical signs in the cat were attributed more to some property of the virus than to the immune response of the cat, although this response and the cat's environmental circumstances were taken into account [106]. This view persisted despite a lack of evidence of either major morphological or serological differences between pathogenic and non-pathogenic strains of FCoV [13].

During the period of the research described in this thesis, as a result of this study and other investigations, the distinctions between FECV and FIPV were eroded. For example, in the survey described in Chapter 4 cases of FCoVV occurred in cats from households which had histories more in keeping with

FECV infection. Workers in Liverpool University found that a cat developed lesions of FCoVV on exposure to CCV [83]. Finally, the proponent of the non-virulent coronavirus changed his views to a belief that all FCoV infections are caused by FECV, with mutants of the virus occurring within individual cats which were able to replicate in the macrophage preferentially to the enterocyte and cause vasculitis [108]. Thus the argument against the IFA test on this basis was largely invalidated.

It seems clear, after the research described in this thesis, that in any household where cats are continually being infected with FCoV, sooner or later deaths will occur. Since the mortality rate is only 3-12%, a household with only a few cats statistically may have to wait some time before a death occurs. However, this does not mean that the virus endemic to that household is any less capable of causing disease. Laboratory strains capable of causing differing disease in cats do exist, though how much they are a product of the passaging system used to grow virus stock and how much they are a reflection of real viral differences in the field, is unknown.

Unfortunately, the actual levels of IFA titres in an individual cat tells one little unless the titre returns to zero, in which case the cat is no longer infectious. In experimental infections, antibody titres classically climb rapidly and sometimes decline towards the end until the development of disease [101, 156, 157]. In natural infections the antibody patterns of the cats which succumbed were indistinguishable from those of cats which still appeared to be healthy. Even some cats which seemed to have recovered and became seronegative for quite some time, died from the damage caused to the blood vessels and the organs they supplied.

Many participants in the survey have agreed to continue to have their cats monitored for a further two years. It is likely that mortality rates due to FCoV will increase as more animals succumb to damage sustained during transient IC-associated

vasculitis. It seems unlikely that any test could be developed to give an accurate prognosis for the individual cat because of the nature of the disease. At best, statistical probabilities might be all we can offer.

A very significant finding of the longitudinal study was the appparent lack of ADE of infection in the field. ADE of FCoV infection is a commonly reported phenomenon in the laboratory and therefore was expected to exist also in natural infections. Cats in this study were presumed to have been reinfected when their IFA titres fell and then rose again but no evidence of ADE was seen. Presumably many have either resistance to infection by local gut or respiratory tract immunity or have systemic CMI. An interesting study would be to examine the cytotoxic T-cell response in these cats.

Total prevention of infection is the obvious course of choice and for this to be achieved, widespread testing of cats by IF before introducing into known seropositive or seronegative households should be promoted.

Another argument against the IFA test arose because of its occasional misuse: healthy cats were destroyed on the basis of a high antibody titre. This hazard caused some to advocate that no testing was the lesser of two evils. Three aspects of the research described in this thesis should hopefully alleviate this situation: the finding that cats in 33% of the households followed became seronegative; that kittens born into seropositive households could be prevented from becoming infected by careful management; and that carrier animals did not necessarily have high IFA titres.

Previously, it was believed that cats would remain seropositive and might be infectious indefinitely. In the absence of a vaccine or test for antigen, there seemed to be no way of eradicating the infection from a household short of clearing out all the cats and starting over again. However, sequential

testing of households shows that many cats do eliminate the infection although it can take up to 3 years or more. Reintroduction of virus to 9 of the 24 households which had become seronegative occurred mainly because of the introduction of an untested cat, showing the importance of continued surveillance using the IFA test.

In the kitten survey, 1 in 3 seropositive cats was found to excrete virus and in the adult survey, cats kept in groups of up to three were more likely to have falling antibody titres. Therefore it would seem that FCoV infection may be maintained by cats being continually reinfected and shedding for a time. If this cycle could be broken, the infection would peter out.

In the past, cat breeders who were found to have cats with anti-FCoV antibodies were often advised to stop breeding their cats either for a time or permanently. The kitten survey showed that this action was not necessary provided the kittens could be isolated from the adult cats in the cattery. This regime would also be beneficial in limiting the spread of other infectious diseases to susceptible kittens.

The existence of the healthy carrier cat has long been postulated [104, 111, 114, 141] and was definitively established in the study of kittens born into catteries followed in this study. Previously it was suggested that cats with higher antibody titres were more likely to be virus shedders than cats with low titres [103, 111]. The present research established that most carriers had low to moderate levels of antibodies. An important finding was that seronegative cats did not shed virus, something which previously was not known and was sometimes used in the anti-IFA argument [133]. Further, one third of carriers became seronegative showing that the carrier status is not necessarily permanent.

During the period of this study it became apparent that many

manifestations of the disease caused by FCoV are not adequately described by the classical descriptions of 'wet' and 'dry' FIP. Cats presented with such diverse clinical signs as Heinz body anaemia, pemphigus, kidney failure and vestibular signs, many of which were seronegative, were found to have their origin in FCoV infection. A more useful approach for the clinician in understanding the disease is to consider its pathogenesis and to remember that the basic lesion is a vasculitis.

The use of immunoblotting in diagnosis of FCoV had not previously been examined. Immunoblotting revealed a difference in anti-M levels in cats which died of FCoVV compared with those which survived infection. Plans for future experiments include devising an IF assay of antibodies against each of the viral structural proteins based on target cells infected with vaccinia virus recombinants expressing S, N or M so that the sera described in Chapter 6 can be re-examined to determine if there is a level of anti-M antibody which indicates a poor prognosis.

Absence of anti-M and anti-S on immunoblotting indicated that a cat was no longer excreting virus, a finding which is useful in the situation where a cat owner wishes to obtain a new cat and needs to know if the existing cat(s) would pose a threat to a newcomer.

In conclusion, it is hoped that the value of the longitudinal field survey has been demonstrated by the research described in this thesis. A bank of sera now exists from naturally-infected cats whose fate is known and these sera are available for future experiments.

On the basis of results presented in this thesis, much more specific advice can now be given to veterinary surgeons in the field about dealing with FCoV antibody positive cats.

References

1. Addie D.D., Jarrett O. 1990 Control of feline coronavirus infection in kittens. Vet. Rec. **126** 164

2. August J.R. 1984 Feline infectious peritonitis. An immune-mediated coronaviral vasculitis. Vet. Clin. of N. America: Small Animal Practice **14** 5 971-984

3. Barlough J.E. Jacobson R.H. Scott F.W. 1983 Feline coronaviral serology. Feline Practice **13** 3 25-35

4. Barlough J.E. Jacobson R.H. Scott F.W. 1983 Microtiter assay for coronavirus-neutralizing activity in cats using a canine continuous cell line (A-72) Lab. Anim. Sci. **33** 6 567-570

5. Barlough J.E., Weiss R.C. 1983 Feline infectious peritonitis. Current Veterinary Therapy VIII. Ed. Kirk 1186-1193

6. Barlough J.E., Jacobson R.H., Pepper C.E., Scott F.W. 1984 Role of recent vaccination in production of false-positive coronavirus antibody titers in cats. J. Clin. Microbiol. **19** 3 442-445

7. Barlough J.E. 1984 Serodiagnostic aids and management practice for feline retrovirus and coronavirus infections. Vet. Clin. of N. America: Small Animal Practice **14** 5 955-969

8. Barlough J.E., Summers B.A. 1984 Encephalitis due to feline infectious peritonitis virus in a twelve-week-old kitten. Feline Practice. **14** 1 43-46

9. Barlough J.E. 1985 Cats, coronaviruses and coronavirus antibody tests. J. Sm. Anim. Prac. **26** 353-362

10. Barlough J.E., Jacobson R.H., Scott F.W. 1985 Effect of recent vaccination on feline coronaviral antibody test results. Feline Practice **15** 5 17-26

11. Barlough J.E., Johnson-Lussenburg C.M., Stoddart C.A., Jacobson R.H., Scott F.W. 1985 Experimental inoculation of cats with human coronavirus 229E and subsequent challenge with feline infectious peritonitis virus. Can. J. Comp. Med. **49** 303-307.

12. Barlough J.E., Jacobson R.H., Sorresso G.P., Lynch T.J., Scott F.W. 1986 Coronavirus antibody detection in cats by computer-assisted kinetics-based enzyme-linked immunosorbent assay (KELA): field studies. Cornell Vet. **76** 227-235

13. Bartkoski M.J. 1990 Blood tests for FIP. Vet.Rec. **126** 272-3

14. Beesley J E., Hitchcock L M. 1982 The ultrastructure of feline infectious peritonitis virus in feline embryonic lung cells. J. Gen. Virol. **59** 23-28

15. Boyle J.F., Pedersen N.C., Evermann J.F., McKeirnan A.J., Ott R.L., Black J.W. 1984 Plaque assay, polypeptide composition and immunochemistry of feline infectious peritonitis virus and feline enteric coronavirus isolates. Adv. Exp. Med. Biol. **173** 133-147

16. Briggs M.B., Evermann J.F., McKeirnan A.J. 1986 Feline infectious peritonitis Feline Practice **16** 2 13-16

17. Callebaut P., Correa I., Pensaert M., Jimenez G., Enjuanes L. 1988 Antigenic differentiation between transmissible gastroenteritis virus of swine and a related porcine respiratory coronavirus. J. Gen. Virol. **69** 1725-1730

18. Cavanagh D., Brian D.A., Enjuanes L., Holmes K.V., Lai M.M.C., Laude H., Siddell S.G., Spaan W., Taguchi F., Talbot P.J. 1990 Recommendations of the coronavirus study group for the nomenclature of the structural proteins, mRNAs, and genes of coronaviruses. Virology **176** 306-307

19. Christianson K.K., Ingersoll J.D., Landon R.M., Pfeffer N.E., Gerber J.D. 1989 Characterization of a temperature sensitive feline peritonitis coronavirus. Arch. Virol. **109** 185-196

20. De Groot R.J., Ter Haar R.J., Horzinek M.C., Van der Zeijst B.A.M. 1987 Intracellular RNAs of the feline infectious peritonitis coronavirus strain 79-1146. J. Gen. Virol. **68** 995-1002

21. De Groot R.J., Maduro J., Lenstra J.A., Horzinek M.C., Van der Zeijst B.A.M., Spaan W.J.M. 1987 cDNA cloning and sequence analysis of the gene encoding the peplomer protein of feline infectious peritonitis virus. J. Gen. Virol. **68** 2639-2646

22. De Groot R.J., Van Leen R.W., Dalderup M.J.M., Vennema H., Horzinek M.C., Spaan W.J.M. 1989 Stably expressed FIPV peplomer protein induces cell fusion and elicits neutralizing antibodies in mice. Virology **171** 493-502

23. Easton N.J. 1990 Blood tests for FIP. Vet. Rec. **126** 150

24. Else R.W., Simpson J.W. 1988 Diagnostic value of exfoliative cytology of body fluids in dogs and cats. Vet. Rec. **123** 70-76.

25. Espinoza L.R. 1983 Assays for circulating immune complexes. Circulating Immune Complexes Ed. L.R. Espinoza and C.K. Osterland. Futura Publishing

Company Inc. New York. 21-49

26. Eugster A.K., Liauw H. 1979 Detection of antibodies to feline infectious peritonitis (FIP) virus in cats using transmissible gastroenteritis virus as antigen and an electronmicroscopic search for FIP virus. The Southwestern Veterinarian **32** 109

27. Evermann J.F. 1986 Feline coronavirus infection of cheetahs. Feline Practice **16** 3 21-30

28. Feldmann B M., Jortner B S. 1964 Case presentation, Clinico-Pathologic Conference. J. Am. Vet. Med. Assoc. **144** 12 1409-1419

29. Fiscus S.A., Rivoire B.L., Teramoto Y.A. 1987 Epitope-specific antibody responses to virulent and avirulent feline infectious peritonitis virus isolates. J. Clin. Microbiol. **25** 8 1529-1534

30. Fiscus S.A., Teramoto Y.A. 1987 Antigenic comparison of feline coronavirus isolates: evidence for markedly different peplomer glycoproteins. J. Virol. **61** 8 2607-2613

31. Fiscus S.A., Teramoto Y.A. 1987 Functional differences in the peplomer glycoproteins of feline coronavirus isolates. J. Virol. **61** 8 2655-2657

32. Garwes D J. 1988 Transmissible gastroenteritis. Vet. Rec. **122** 462-463

33. Gaskell R. personal communication.

34. Gerber J.D., Ingersoll J.D., Gast A.M., Christianson K.K., Selzer N.L., Landon R.M., Pfeiffer N.E., Sharpee R.L., Beckenhauer W.H. 1990 Protection against feline

infectious peritonitis by intranasal inoculation of a temperature-sensitive FIPV vaccine. Vaccine **8** 536-542

35. Gerber J.D., Pfeiffer N.E., Ingersoll J.D., Christianson K.K., Landon R.M., Selzer N.L., Beckenhauer W.H. 1990 Characterization of an attenuated temperature sensitive feline infectious peritonitis vaccine virus. Adv. Exp. Med. Biol. **276** 481-489

36. Goitsuka R., Hirota Y., Hasegawa A., Tomoda I. 1987 Release of interleukin 1 from peritoneal exudate cells of cats with feline infectious peritonitis. Jpn. J. Vet. Sci. **49** (5) 811-818

37. Goitsuka R., Onda C., Hirota Y., Hasegawa A., Tomoda I. 1988 Feline interleukin 1 production induced by feline infectious peritonitis virus. Nippon Jugaku Zasshisd **50** 1 209-214

38. Goitsuka R., Ohashi T., Ono K., Yasukawa K., Koishibara Y., Fukui H., Ohsugi Y., Hasegawa A. 1990 IL-6 activity in feline infectious peritonitis. J. Immunol. **144** 2599-2603

39. Goitsuka R., Tsuji M., Ohashi T., Onda C., Tuchiya K., Hirota Y., Takahashi E., Hasegawa A. 1991 Characterization of a feline infectious peritonitis virus isolate. J. Vet. Med. Sci. **53** (2) 337-339

40. Goitsuka R., Furusawa S., Mizoguchi M., Hasegawa A. 1991 Detection of Interleukin 1 in ascites from cats with feline infectious peritonitis. J. Vet. Med. Sci. **53** (3) 487-489

41. Gorman N.T., Werner L.L. 1986 Diagnosis of immune-mediated diseases and interpretation of immunologic tests. Current Veterinary Therapy IX. Ed. Kirk 427-435

42. Gouffaux M., Pastoret P.P., Henroteux M., Massip A. 1975 Feline infectious peritonitis proteins of plasma and ascitic fluid. Vet. Pathol. **12** 335-348

43. Hardy W.D. Jr., Hurvitz A.I. 1971 Feline infectious peritonitis: experimental studies. J. Am. Vet. Med. **158** 6 994-1002

44. Harvey J.W., Gaskin J.M. 1978 Feline haptoglobin. Am. J. Vet. Res. **39** 4 549-553

45. Hayashi T., Goto N., Takahashi R., Fujiwara K. 1978 Detection of coronavirus-like particles in a spontaneous case of feline infectious peritonitis. Jpn. J. Vet. Sci. **40** 207-212

46. Hayashi T., Doi K., Fujiwara K. 1984 Role of circulating antibodies and thymus-dependent lymphocytes in production of effusive type feline infectious peritonitis after oral infection. Adv. Exp. Med. Biol. **173** 383-4

47. Hayashi T., Ishida T., Fujiwara K. 1982 Glomerulonephritis associated with feline infectious peritonitis Jpn. J. Vet. Sci. **44** (6) 909-916

48. Hayashi T., Watabe Y., Nakayama H., Fujiwara K. 1982 Enteritis due fo feline infectious peritonitis virus Jpn. J. Vet. Sci. **44** (6) 97-907

49. Hayashi T., Watabe Y., Takenouchi T., Fujiwara K. 1983 Role of circulating antibodies in feline infectious peritonitis after oral infection. Jpn. J. Vet. Sci. **45** 4 487-494

50. Hayashi T., Sasaki N., Ami Y., Fujiwara K. 1983 Role of thymus-dependent lymphocytes and antibodies in feline

infectious peritonitis after oral infection. Jpn. J. Vet. Sci. **45** 6 759-766

51. Hayashi T., Yamakoshi J., Ami Y., Fujiwara K. 1984 Role of neutrophils in production of early enteritis after oral infection with feline infectious peritonitis virus in passively sensitized kittens. Jpn. J. Vet. Sci. **46** 3 273-279

52. Hirano T., Akira S., Taga T., Kishimoto T. 1990 Biological and clinical aspects of interleukin 6. Immunol. Today **11** 12 443-449

53. Hitchcock L.M., O'Reilly K. J., Beesley J.E. 1981 In vitro culture of feline infectious peritonitis virus. Vet. Rec. **108** 535-537

54. Hohdatsu T., Sasamoto T., Okada S., Koyama H. 1991 Antigenic analysis of feline coronaviruses with monoclonal antibodies (MAbs): preparation of MAbs which discriminate between FIPV strain 79-1146 and FECV strain 79-1683. Vet. Microbiol. **28** 13-24

55. Holzworth J. 1963 Some important disorders of cats. Cornell Vet. **53** 157-160

56. Horzinek M.C., Osterhaus A.D.M.E. 1979 Feline infectious peritonitis: a worldwide serosurvey. Am. J. Vet. Res. **40** 1487 - 1492

57. Horzinek M.C., Osterhaus A.D.M.E. 1979 The virology and pathogenesis of feline infectious peritonitis. Arch. Virol. **59** 1-15

58. Horzinek M.C., Lutz H., Pedersen N.C. 1982 Antigenic relationships among homologous structural polypeptides of porcine, feline and canine coronaviruses. Infection

and Immunity **37** 3 1148-1155

59. Horzinek M.C., Ederveen J., Egberink H., Jacobse-Geels H.E.L., Niewold T., Prins J. 1986 Virion polypeptide specificity of immune complexes and antibodies in cats inoculated with feline infectious peritonitis virus. Am. J. Vet. Res. **47** 4 754-761

60. Hoshino Y., Scott F.W. 1978 Brief communication: replication of feline infectious peritonitis virus in organ cultures of feline tissue. Cornell Vet. **68** 411-417

61. Hoshino Y., Scott F.W. 1980 Coronavirus-like particles in the feces of normal cats. Arch. Virol. **63** 147-152

62. Hoshino Y., Scott F.W. 1980 Immunofluorescent and electron microscopic studies of feline small intestinal organ cultures infected with feline infectious peritonitis virus. Am. J. Vet. Res. **41** 5 672-681

63. Hosie M.J., Robertson C., Jarrett O. 1989 Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. Vet. Rec. **128** 293-297

64. Hoskins J.D. 1991 Coronavirus infection in cats. The Compendium: Small Animal. **13** 4 567-586

65. Ingersoll J.D., Wylie D.E. 1988 Identification of viral antigens that induce antibody responses on exposure to coronaviruses. Am. J. Vet. Res. **49** 9 1467 - 1471

66. Ingersoll J.D., Wylie D.E. 1988 Comparison of serologic assays for measurement of antibody response to coronavirus in cats. Am. J. Vet. Res. **49** 9 1472-1478

67. Ishida T., Washizu T., Fukuoka J., Toriyabe K., Uchino T.,

Motoyoshi S. 1987 Feline infectious peritonitis virus antibody test using enzyme-linked immunosorbent assay. Jpn. J. Vet. Sci. **49** 1 145-149

68. Ishida T., Fukuoka J., Toriyabe K., Motoyoshi S. 1987 Serodiagnosis of feline infectious peritonitis. Adv. Exp. Med. Biol. **218** 577-578

69. Ishida T., Washizu T., Toriyabe K., Motoyoshi S., Tomoda I., Pedersen N.C. 1989 Feline immunodeficiency virus infection in cats in Japan. J. Am. Vet. Med. Assoc. **194** 2 221-225

70. Jacobse-Geels H.E.L., Daha M.R., Horzinek M.C. 1980 Isolation and characterization of feline C3 and evidence for the immune complex pathogenesis of feline infectious peritonitis. J. Immunol. **125** 4 1606-1610

71. Jacobse-Geels H.E.L., Daha M.R., Horzinek M.C. 1982 Antibody, immune complexes, and complement activity fluctuations in kittens with experimentally · induced feline infectious peritonitis virus. Am. J. Vet. Res. **43** 4 666-670

72. Jacobse-Geels H.E.L., Horzinek M.C. 1983 Expression of feline infectious peritonitis coronavirus antigens on the surface of feline macrophage-like cells. J. Gen. Virol. **64** 1859-1866

73. Jacobse-Geels H.E.L., Horzinek M.C. 1984 Expression of feline infectious peritonitis (FIP) coronavirus antigens on the surface of feline macrophage-like cells. Adv. Exp. Med. Biol. **173** 381-382

74. Jarrett, O., Laird, H.M., and Hay, D. 1973 Determinants of the host range of feline leukaemia virus. J. Gen. Virol. **20** 169-175

75. Jarrett, O., Golder M.C., and Stewart M.F. 1982 Detection of transient and persistent feline leukaemia virus infections. Vet. Rec. **110** 225-228

76. Jarrett, O. 1987 Personal communication.

77. Kitchen L.W. 1988 Effect of diethylcarbamazine on serum antibodies to feline infectious peritonitis in cats. Vaccine **6** 10-11

78. Krum S., Johnson K., Wilson J. 1975 Hydrocephalus associated with the noneffusive form of feline infectious peritonitis. J. Am. Vet. Med. Assoc. **167** 8 746-748

79. Loeb W.F. 1970 Laboratory finding in feline infectious peritonitis. Mod. Vet. Pract. **51** 23

80. Loeffler D.G., Ott R.L., Evermann J.F., Alexander J.E. 1978 The incidence of naturally occurring antibodies against feline infectious peritonitis in selected cat populations. Feline Practice **8** 1 43 - 47

81. Lutz H., Hauser B., Horzinek M.C. 1986 Feline infectious peritonitis (FIP) - the present state of knowledge. J. Sm. Anim. Prac. **27** 108-116

82. McArdle F. 1991 PhD thesis: Studies on feline infectious peritonitis. Liverpool University

83. McArdle F., Bennett M., Gaskell R.M., Tennant B., Kelly D.F., Gaskell C.J. 1990 Canine coronavirus infection in cats; a possible role in feline infectious peritonitis. Adv. Exp. Med. Biol. **276** 475-9.

84. McIntosh K. 1974 Coronaviruses: a comparative review. Current Topics in Microbiology and Immunology. **63** 85-93

85. McKeirnan A.J., Evermann J.F., Hargis A., Ott R.L. 1981 Isolation of feline coronaviruses from two cats with diverse disease manifestations. Feline Practice. **11** 3 16-20

86. McKeirnan A.J., Evermann J.F., Davis E.V., Ott R.L. 1986 Comparative properties of feline coronaviruses in vitro. Can. J. Vet. Res. **51** 212-216

87. Montali R.J., Strandberg J.D. 1972 Extraperitoneal lesions in FIP. Vet. Path. **9** 109-121

88. Nafe L.A. 1984 Feline infectious peritonitis. Vet. Clin. of N. America **14** 6 1295-1297

89. Norsworthy G.D. 1974 Neonatal Feline Infectious Peritonitis. Feline Practice. Nov.-Dec. 1974 34

90. Norsworthy G.D. 1979 Kitten mortality complex. Feline Practice **9** 2 57-60

91. O'Brien S.J., Roelke M.E., Marker L., Newman A., Winkler C.A., Meltzer D., Colly L., Evermann J.F., Bush M., Wildt D.E. 1987 Genetic basis for species vulnerability in the cheetah. Science. **227** 1428-1434

92. Okoshi S., Tomodo I., Makimura S. 1967 Analysis of normal cat serum by immunoelectrophoresis. Jpn. J. Vet. Sci. **29** 337-345

93. O'Reilly K.J., Fishman B., Hitchcock L.M. 1979 Feline infectious peritonitis: isolation of a coronavirus. Vet. Rec. **104** 348

94. Osterhaus A.D.M.E., Horzinek M.C., Reynolds D.J. 1977 Seroepidemiology of feline infectious peritonitis virus infections using transmissible gastroenteritis virus as

antigen. Zbl. Vet. Med. B. **24** 835-841

95. Osterhaus A.D.M.E. La peritonite infectieuse feline: une infection a coronavirus du chat. Ann. Med. Vet. **128** 169-175

96. Ott R.L. 1986 Diagnosis of feline viral infection. Vet. Clin. of N. America. **16** 6 1157-1170

97. Pastoret P.P., Gouffaux M., Henroteaux M. 1974 Description et etude experimentale de la peritonite infectieuse feline. Ann. Med. Vet. **118** 479-492

98. Pastoret P.P., Gouffaux M., Henroteaux M., Schoenaers F. 1977 Feline infectious peritonitis virus. J. Am. Vet. Med. Assoc. **171** 8 740-741

99. Pastoret P.P., Henroteaux M. 1978 Epigenetic transmission of feline infectious peritonitis. Comp. Immum. Microbiol. Infect. Dis. 1 67-70

100. Pedersen N.C. 1976 Morphologic and physical characteristics of feline infectious peritonitis and its growth in autochthonous peritoneal cell cultures. Am. J. Vet. Res. **37** 567-572

101. Pedersen N.C. 1976 Feline infectious peritonitis: something old, something new. Feline Practice May 1976 42-51

102. Pedersen N.C. 1976 Serologic studies of naturally occurring feline infectious peritonitis. Am. J. Vet. Res. **37** 12 1449-1453

103. Pedersen N.C. 1983 Feline infectious peritonitis and feline enteric coronavirus infections. Part 1. Feline Practice **13** 4 13-19

104. Pedersen N.C. 1983 Feline infectious peritonitis and feline enteric coronavirus infections. Part 2. Feline Practice **13** 5 5-20

105. Pedersen N.C. 1984 Feline coronavirus infections. Clinical Microbiology and Infectious Diseases of the Dog and Cat. Ed. Greene. W.B. Saunders Co. 514-526

106. Pedersen N.C. 1985 Feline infectious peritonitis. Comparative Pathobiology of Viral Diseases Vol. II Ed. Olsen R.G., Krakowka S. & Blakeslee J.R. Jr. CRC Press. 115-136

107. Pedersen N.C. 1989 Animal virus infections that defy vaccination: equine infectious anaemia, caprine arthritis-encephalitis, Maedi-Visna, and feline infectious peritonitis. Adv. Vet. Sci. and Comp. Med. **33** 413-428

108. Pedersen N.C. 1991 Interpretation of feline infectious peritonitis virus serology. BSAVA Congress 1991 Paper Synopses 17

109. Pedersen N.C., Boyle J.F. 1980 Immunologic phenomena in the effusive form of feline infectious peritonitis. Am. J. Vet. Res. **41** 868-876

110. Pedersen N.C., Boyle J.F., Floyd K. 1981 Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. Am. J. Vet. Res. **42** 3 363-367

111. Pedersen N.C., Boyle J.F., Floyd K., Fudge A., Barker J. 1981 An enteric coronavirus infection of cats and its relationship to feline infectious peritonitis. Am. J. Vet. Res. **42** 3 368-376

112. Pedersen N.C. Black J.W. 1983 Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus. Am. J. Vet. Res. **44** 2 229-234

113. Pedersen N.C. Black J.W. Boyle J.F. Evermann J.F. McKiernan A.J., Ott R.L. 1984 Pathogenic differences between various feline coronavirus isolates. Adv. Exp. Med. Biol. **173** 365-380

114. Pedersen N.C., Floyd K. 1985 Experimental studies with three new strains of feline infectious peritonitis virus: FIPV-UCD2, FIPV-UCD3, and FIPV-UCD4. The Compendium of Continuing Education **7** 12 1001-1011

115. Pedersen N.C., Johnson L., Ott R.L. 1985 Evaluation of a commercial feline leukemia vaccine for immunogenicity and efficacy. Feline Practice **15** 6 7-20

116. Potkay S., Bacher J.D., Pitts T.W. 1974 Feline infectious peritonitis in a closed breeding colony. Lab. Anim. Sci. **24** 2 279-289

117. Reinacher M., Theilen G. 1987 Frequency and significance of feline leukemia virus infection in necropsied cats. Am. J. Vet. Res. **48** 6 939-945

118. Reinacher M. 1989 Diseases associated with spontaneous feline leukemia virus (FeLV) infection in cats. Vet. Immunol. and Immunopath. **21** 85-95

119. Reynolds D J., Garwes D J., Gaskell C J. 1977 Detection of transmissible gastroenteritis virus neutralising antibody in cats. Arch. Virol. **55** 77-86

120. Roitt I. 1988 Essential Immunology. Sixth edition. Blackwell Scientific Publications.

121. Roitt I., Brostoff J., Male D. 1989 Immunology. Second edition. Gower Medical Publishing.

122. Saif L.J., Bohl E.H. 1986 Transmissible gastroenteritis. Diseases of swine. Sixth edition. Iowa State University Press. 255-274

123. Sanchez C.M., Jimenez G., Laviada M.D., Correa I., Sune C., Bullido M.J., Gebauer F., Smerdou C., Callebaut P., Escribano J.M., Enjuanes L. 1990 Antigenic homology among coronaviruses related to transmissible gastroenteritis virus. Virology **174** 410-417

124. Schwendenwein I., Koelbl S. 1991 Evaluation of a commercially available CELISA test kit for the detection of antibodies against feline infectious peritonitis. Wien. Tieraerztl Monatsschr. **78** 6 177-180

125. Scott F.W., Weiss R.C., Post J.E., Gilmartin J.E., Hoshino Y. 1979 Kitten mortality complex (Neonatal FIP?) Feline Practice **9** 2 44-56

126. Scott F.W. 1986 Feline infectious peritonitis and other feline coronaviruses. Current Veterinary Therapy IX Kirk. W.B. Saunders Co. 1059-1062

127. Scott F.W. 1987 Immunization against feline coronaviruses. Adv. Exp. Med. Biol. **218** 569-578

128. Shelly, S.M., Scarlett-Kranz J., Blue J.T. 1988 Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. J. Am. Anim. Hosp. Assoc. **24** 495-500

129. Shelton G.H., Grant C.K., Cotter S.M., Gardner M.B., Hardy W.D. Jr., DiGiacomo R.F. 1990 Feline immunodeficiency virus and feline leukemia virus infections and their

relationships to lymphoid malignancies in cats: a retrospective study (1968-1988) J. Acquired Immune Deficiency Syndromes **3** 623-630

130. Shockley L.J., Kapke P.A., Lapps W., Brian D.A., Potgieter L.N.D., Woods R. 1987 Diagnosis of porcine and bovine enteric coronavirus infections using cloned cDNA probes. J. Clin. Microbiol. **25** 9 1591-1596

131. Siddell S., Wege H., Meulen V Ter. 1982 The structure and replication of coronaviruses. Curr. Top. Microbiol. Immun. **99** 131-160

132. Siddell S., Wege H., Meulen V Ter., 1983 The biology of coronaviruses. J. Gen. Virol. **64** 761-776

133. Sparkes A.H., Gruffydd-Jones T.J., Harbour D.A., Hopper C.D., Smerdon T.N. 1990 Blood tests for FIP. Vet. Rec. **126** 225

134. Sparkes A.H., Gruffydd-Jones T.J., Harbour D.A., 1991 Feline infectious peritonitis: a review of clinicopathological changes in 65 cases, and a critical assessment of their diagnostic value. Vet. Rec. **129** 209-212

135. Stoddart C.A., Barlough J.E., Baldwin C.A., Scott F.W. Immunization against feline infectious peritonitis. Submitted to Am. J. Vet. Res.

136. Stoddart C. A., Barlough J. E., Scott F. W. 1984 Experimental studies of a coronavirus and coronavirus-like agent in a barrier-maintained feline breeding colony. Arch. Virol. **79** 85-94

137. Stoddart C. A., Scott F. W. 1988 Isolation and identification of feline peritoneal macrophages for in

vitro studies of coronavirus-macrophage interactions. J. Leukocyte Biology **44** 319-328

138. Stoddart C.A., Barlough J.E., Baldwin C.A., Scott F. W. 1988 Attempted immunization of cats against feline infectious peritonitis using canine coronavirus. Res. Vet. Sci. **45** 383-388

139. Stoddart C.A., Scott F.W. 1989 Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with in vivo virulence. J. Virol. **63** 1 436-440

140. Stoddart M.E. 1985 PhD thesis: Some studies on the pathogenesis of feline infectious peritonitis. Bristol University.

141. Stoddart M.E., Gaskell R.M., Harbour D.A., Gaskell C.J. 1988 Virus shedding and immune responses in cats inoculated with cell culture-adapted feline infectious peritonitis virus. Vet. Microbiol. **16** 145-158

142. Stoddart M.E., Whicher J.T., Harbour D.A. 1988 Cats inoculated with feline infectious peritonitis virus exhibit a biphasic acute phase plasma protein response. Vet. Rec. **123** 621-624

143. Stoddart M.E., Gaskell R.M., Harbour D.A., Pearson G.R. 1988 The sites of early viral replication in feline infectious peritonitis. Vet. Microbiol. **18** 259-272

144. Sturman L.S. 1977 Characterization of a coronavirus. 1. Structural proteins: effects of preparative conditions on the migration of protein in polyacrylamide gels. Virology **77** 637-649

145. Takenouchi T., Ami Y., Hayashi T., Fujiwara K. 1985 Role

of T cells in feline infectious peritonitis virus infection of suckling mice. Jpn. J. Vet. Sci. **47** 465-468

146. Tupper G.T., Evermann J.F., Russell R.G., Thouless M.E. 1987 Antigenic and biological diversity of feline coronaviruses: feline infectious peritonitis and feline enteritis virus. Arch. Virol. **96** 29-38

147. Van Kruiningen H.J., Ryan M.J., Shindel N.M. 1983 The classification of feline colitis. J. Comp. Path. **93** 275-294

148. Vennema H., Heijnen L., Zijderveld A., Horzinek M.C., Spaan W.J.M. 1990 Intracellular transport of recombinant coronavirus spike proteins: implications for virus assembly. J. Virol. **64** 1 339-346

149. Vennema H., de Groot R.J., Harbour D.A., Dalderup M., Gruffydd-Jones T., Horzinek M.C., Spaan W.J.M. 1990 Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization. J. Virol. **64** 3 1407-1409

150. Vennema H., de Groot R.J., Harbour D.A., Dalderup M., Gruffydd-Jones T., Horzinek M.C., Spaan W.J.M. 1990 Immunogenicity of recombinant feline infectious peritonitis virus spike protein in mice and kittens. Adv. Exp. Med. Biol. **276** 217-222

151. Vennema H., de Groot R.J., Harbour D.A., Horzinek M.C., Spaan W.J.M. 1991 Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. Virology. **181** 327-335

152. Ward J.M., Munn R.J., Gribble D.H., Dungworth D.L. 1968

An observation of feline infectious peritonitis. Vet. Rec. **83** 416-417

153. Weiss R.C. 1978 Feline infectious peritonitis - an update. Mod. Vet. Prac. **59** 832-836

154. Weiss R.C., Dodds J.W., Scott F.W. 1980 Disseminated intravascular coagulation in experimentally induced feline infectious peritonitis. Am. J. Vet. Res. **41** 5 663-671

155. Weiss R.C., Scott F.W. 1980 Laboratory diagnosis of feline infectious peritonitis. Feline Practice. **10** 2 16-22

156. Weiss R.C., Scott F.W. 1981 Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with Dengue haemorrhagic fever. Comp. Immun. Microbiol. Dis. **4** 2 175-189

157. Weiss R.C., Scott F.W. 1981 Pathogenesis of feline infectious peritonitis: nature and development of viraemia. Am.J.Vet.Res. **42** 3 382-390

158. Weiss R.C., Scott F.W. 1981 Pathogenesis of feline infectious peritonitis: pathologic changes and immunofluorescence Am. J. Vet. Res. **42** 12 2036-2048

159. Weiss R.C., Cox N.R. 1988 Delayed-type hypersensitivity responses associated with feline infectious peritonitis in two cats. Res. Vet. Sci. **44** 396-398

160. Weiss R.C., Tovio-Kinnucan M. 1988 Inhibition of feline infectious peritonitis virus replication by recombinant human leukocyte (alpha) interferon and feline fibroblastic (beta) interferon. Am. J. Vet. Res. **49** 8 1329-1335

161. Weiss R.C., Cox N.R. 1989 Evaluation of immunity to feline infectious peritonitis in cats with cutaneous viral-induced delayed hypersensitivity. Vet. Immunol. and Immunopath. **21** 293-309

162. Weiss R.C. 1991 The diagnosis and clinical management of feline infectious peritonitis. Vet. Med. **86** 3 308-319

163. Winston S., Fiscus S., Hesterberg L., Matsushita T., Mildbrand M., Porter J., Teramoto Y. 1987 Vet. Immunol. and Immunopath. **17** 453-464

164. Woods R.D. 1982 Studies on enteric coronaviruses in a feline cell line. Vet. Microbiol. **7** 427-435